Chemical Name: Substituted p-Phenylenediamines Category
Submitter: ACC

Chemicals within Category:

CHEMICAL NAME	CASRN
Alkylated N-PPD	
p-Phenylenediamine, N,N-di-sec-butyl	101-96-2
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl)	3081-14-9
4-Aminodiphenylamine Derivatives	
p-Phenylenediamine, N-Isopropyl-N'-phenyl-,	*101-72-4**
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl	*793-24-8**
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives	68953-84-4
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl	3081-01-4
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl	15233-47-3

*These chemicals were used to support conclusions reached for the category

As the Agency received data from High Production Challenge Program participants, it posted notice of and links to those data here for public review and comment. Companies and consortia were requested to defer any proposed new testing on their chemicals for a period of 120 days from when their Test Plans and Robust Summaries were posted to the Internet, in order to allow for technical public comment regarding the possible provision of additional existing data or other technical information which might address or eliminate the need for some new testing.

Some sponsors of chemicals submitted revised test plans and robust summaries to the Agency and referred to them as "final" submissions. EPA previously referred to the most recent submission as "revised" and has made no distinction or judgment whether a submission is final. Lastly, technical public comments on test plans and robust summaries were also provided for several chemicals/categories.

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The pdf contains the following documents:

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council
Good Chemistry Makes It Possible

December 13, 2001

Via US Mail and e-mail

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency (EPA)
P.O. Box 1473
Merrifield, VA 22116

Re: Rubber and Plastic Additives (RAPA) Panel, Consortium No. 1101108
HPV Chemical Challenge Program Submission
Substituted p-Phenylenediamines (PPD) Category
Category Justification and Testing Rationale

Dear Governor Whitman:

The RAPA Panel of the American Chemistry Council is pleased to submit the subject documents to EPA's HPV Chemical Challenge Program (Program) as our test plan for a category covering five of the 39 chemicals RAPA is voluntarily sponsoring in the Program. The RAPA Panel includes the following member companies: Bayer Corporation, Ciba Specialty Chemicals Corporation, Crompton Corporation, Flexsys America L.P., The Goodyear Tire & Rubber Company, The Lubrizol Corporation, Noveon, Inc., R.T. Vanderbilt Company, Inc., and UOP, LLC.

In this submission, please find the *Category Justification and Testing Rationale* for the category *Substituted p-Phenylenediamines*. Five chemicals in the category are sponsored in the Program, as listed in the following table:

RAPA Panel Substituted p-Phenylenediamines Category Chemicals Sponsored in the US HPV Chemical Challenge Program	
CAS Number	**Compound Name**
101-96-2	p-Phenylenediamine, N,N-di-sec-butyl
3081-14-9	p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl)
68953-84-4	1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives
3081-01-4	p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl
15233-47-3	p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl,

Data for two additional chemicals in the category, listed in the table below, are used to support the conclusions reached for the category.

RAPA Panel Substituted p-Phenylenediamines Category Additional Chemicals in the Category	
CAS Number	**Compound Name**
101-72-4	p-Phenylenediamine, N-Isopropyl-N'-phenyl-,
793-24-8	p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl

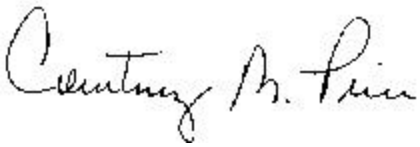
In addition to the *Category Justification and Testing Rationale*, please also find attached robust summaries contained in IUCLID-formatted documents for each of the five sponsored chemicals and the two supporting chemicals in the category.

This submission is also being sent electronically to the following e-mail addresses:

Oppt.ncic@epa.gov
Chem.rtl@epa.gov

If you require additional information, please contact the RAPA Panel's technical contact, Dr. Anne P. LeHuray at (703) 741-5630 or *anne_lehuray@americanchemistry.com*.

Sincerely yours,



Courtney M. Price
Vice President, CHEMSTAR

Attachments

Cc: C. Auer, EPA/OPPT
B. Leczynski, EPA/OPPT
RAPA Panel (without attachments)
S. Russell, ACC (without attachments)

Substituted p-Phenylenediamines
Category Justification and Testing Rationale

CAS Nos. 101-96-2, 3081-14-9, 3081-01-4, 15233-47-3, and 68953-84-4
(+ SIDS Chemicals 101-72-4 and 793-24-8 for data purposes)
Rubber and Plastic Additives Panel
American Chemistry Council
December 2001

List of Member Companies in the Rubber and Plastic Additives Panel

The Rubber and Plastic Additives Panel of the American Chemistry Council include the following member companies: Bayer Corporation, Ciba Specialty Chemicals Corporation, Crompton Corporation, Flexsys America L.P., The Goodyear Tire & Rubber Company, The Lubrizol Corporation, Noveon, Inc., R.T. Vanderbilt Company, Inc., and UOP, LLC.

Executive Summary

The American Chemistry Council's Rubber and Plastic Additives Panel (RAPA), and its member companies, hereby submit for review and public comment their test plan for the Substituted p-Phenylene diamines category of chemicals under the Environmental Protection Agency's High Production Volume (HPV) Challenge Program.

As discussed in the report that follows, Substituted p-Phenylenediamines (PPD), which are used as antidegradants in rubber, fuel additives, or in monomer distillation, are defined as phenylenediamines with various substitutions. These uses require stability at high temperatures, low biodegradation and very low water solubility and low vapor pressure. In consideration of animal welfare concerns to minimize the use of animals in the testing of chemicals, the Panel has conducted a thorough literature search for all available data, published and unpublished. It has also performed an analysis of the adequacy of the existing data. Further, it developed a scientifically supportable category of related chemicals and used structure-activity relationship information to address certain data requirements. Existing data for members of this category indicate that they are of moderate to high toxicity in the aquatic environment, and of low concern for mammalian toxicity. No testing is proposed for the chemicals that constitute the Substituted p-Phenylenediamines category for the purposes of the HPV Program.

Substituted p-Phenylenediamines category

Relying on several factors specified in EPA's guidance document on "Development of Chemical Categories in the HPV Challenge Program," in which use of chemical categories is encouraged, the following closely related chemicals constitute a chemical category:

Substituted p-Phenylenediamines

Alkylated PPD

p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)

4-Aminodiphenylamine Derivatives

p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3)

The goal of developing a chemical category is to use interpolation and/or extrapolation to assess chemicals rather than conducting additional testing with specific consideration of animal welfare concerns to minimize the use of animals in the testing of chemicals.

Structural Similarity. A key factor supporting the classification of these chemicals as a category is their structural similarity (see Figure 1). All materials in this category are phenylenediamines with various substituent groups that are always in the *para* position of the aromatic ring. The substituent groups may be all alkyl, all aryl, or mixed alkyl/aryl.

Similarity of Physicochemical Properties. The similarity of the physicochemical properties of these materials parallels their structural similarity. All are highly-colored (dark brown, purple, reddish or black) solids or semi-viscous liquids intended for use as antidegradants in dark-colored or black finished rubber articles or functional fluids. The use of these materials requires that they be stable under high temperatures. Their low volatility is due to their low vapor pressure, semi-viscous or solid form. The existing information for these materials indicates that they have very low water solubility and high flash points.

Fate and Transport Characteristics. Members of this category have been tested and shown not to be readily biodegradable via CO2 evolution, but they are susceptible to both hydrolysis and photodegradation. Additional data collection efforts are not necessary. These materials have been shown not to partition to water or air if released into the environment due to their low water solubility and low vapor pressure; as a result additional computer-modeled environmental partitioning data is not necessary for the members of this category, for the purposes of the HPV Program.

Toxicological Similarity. Review of existing published and unpublished test data for Substituted p-Phenylenediamines shows the aquatic and mammalian toxicity among the materials within this category are similar.

> **Aquatic Toxicology**. Data on acute fish toxicity, acute invertebrate toxicity, and algae toxicity were reviewed. The Substituted p-Phenylenediamines, in general, are very toxic to aquatic organisms. Additional testing is not proposed for these materials for the purposes of the HPV Program.

Mammalian Toxicology - Acute. Data on acute mammalian toxicity were reviewed, and the findings indicate a low concern for acute toxicity for all materials. Data are available for most members of the category indicating that the category has been well tested for acute mammalian effects. Therefore, no additional acute mammalian toxicity testing is proposed for the purposes of the HPV Program.

Mammalian Toxicology - Mutagenicity. Data from bacterial reverse mutation assays, *in vitro* and *in vivo* chromosome aberration studies, as well as additional supporting *in vitro* and *in vivo* genetic toxicity studies were reviewed, and the findings indicate a low concern for mutagenicity. Data are available for several members of the category or close structural analogs, and these data can be bridged to the other members of the category. Therefore, the category has been adequately tested for mutagenicity to meet the requirements of the HPV Program; therefore, no additional mutagenicity testing is proposed.

Mammalian Toxicology – Repeated Dose Toxicity. Data from repeated-dose toxicity studies were reviewed and sufficient data are available to satisfy the repeated dose toxicity requirements of this category through bridging to members without test data, such that additional testing is not proposed for these materials for the purposes of the HPV Program.

Mammalian Toxicology - Reproductive and Developmental Toxicity. There are several adequate reproductive/developmental studies for members of the Substituted p-Phenylene diamines category. Again, existing study data and results can be bridged to other category members, such that additional testing is not proposed for the purposes of the HPV Program.

Conclusion. Based upon data reviewed for the HPV program, the physicochemical and toxicological properties of the proposed Substituted p-Phenylenediamines category members are similar and follow a regular pattern as a result of that structural similarity. Therefore, the EPA definition of a chemical category has been met. Further, the availability and results of data for the chemicals that constitute the Substituted p-Phenylenediamines category indicate that no additional testing needs to be conducted for the purposes of the HPV Program.

Introduction

A provision for the use of structure activity relationships (SAR) to reduce testing needs is included under EPA's HPV Program. Specifically, categories may be formed based on structural similarity, through analogy, or through a combination of category and analogy for use with single chemicals. The benefits of using a category approach are numerous and include accelerated release of hazard information to the public (category analysis and testing are proposed to be initiated within the first two years of the HPV Program); reduction in the number of animals used for testing; and an economic savings as a result of a reduced testing program.

The Substituted p-Phenylenediamines that form this category based on structural similarity are:

Alkylated N-PPD
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)

4-Aminodiphenylamine Derivatives
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3)

The category has been arranged into two primary subcategories (Alkylated N-PPD and 4-Aminodiphenylamine Derivatives) for purposes of bridging data to the closest related material. The materials were further arranged in order of molecular weight, so that the smallest material is listed first, and the following materials have increasingly larger molecular weights. Of these, p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (CAS#101-72-4) has been evaluated in the Organization for Economic Co-operation and Development (OECD) Screening Information Data Set (SIDS) program and p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (CAS#793-24-8) is currently in the OECD SIDS evaluation process. Data for these two members of the Substituted p-Phenylene diamines category are included in support of the five category members sponsored in the HPV Program.

The development of this category follows current EPA guidance[1].

Background Information: Manufacturing and Commercial Applications

Manufacturing

Substituted p-Phenylenediamines are manufactured batchwise in high-pressure autoclave reactors using a process known as catalytic reduction. In a typical reaction process, the chemical intermediate 4-Aminodiphenylamine (CAS#101-54-2) is reacted with the appropriate ketone and hydrogen gas in the presence of a precious metal catalyst on carbon to form the product, which is then purified via separation, filtration and azeotropic distillation.

Commercial Applications

In the U.S., Substituted p-Phenylenediamines are used primarily as antidegradants in the production of black or dark-colored rubber, as fuel additives and in monomer distillation processes. They are widely used in the manufacture of tires (sidewall, tread and retread, carcass, belt skim, liner, bead filler/chafer, and base tread), moldings, hoses, belts and gaskets for the automotive industry and in other industrial rubber products such as roofing material that are exposed to the elements. Others are used as fuel additives to prevent air oxidation, and a few find usage as “short-stoppers” or polymerization inhibitors in the process of monomer distillation. Substituted p-Phenylenediamines are powerful antioxidants/antiozonants that greatly extend the useful life of

[1] US EPA, Office of Pollution Prevention and Toxics. Development of Chemical Categories, Chemical Right-to-Know Initiative. http://www.epa.gov/opptintr/chemrtk/categuid.htm

rubber articles and functional fluids by delaying the oxidative aging process. These highly-colored, or "staining" antidegradants also help prevent surface cracking due to flex fatigue in dynamic applications. Typical usage level for the Substituted p-Phenylenediamines in these industrial applications ranges from 0.5 – 3%.

FDA Status – The Substituted p-Phenylenediamines are not widely used in food contact applications due to their capability to stain and discolor. However, two chemicals in this category have some limited food-contact applications:

175.105	Components of Adhesives	68953-84-4
177.2600	Rubber Articles	68953-84-4 and 101-72-4

Shipping/Distribution

Substituted p-Phenylenediamines are shipped extensively throughout the world from manufacturing plants located in North and South America, Eastern and Western Europe, China and Japan. These materials are typically shipped by tank car, tank truck, and barge.

Worker/Consumer Exposure

The rubber and plastics additives industry has a long safety record and sophisticated industrial users handle materials. Exposure of workers handling PPD category chemicals is likely to be the highest in the area of material packaging rather than manufacturing. These materials are made as pastilles (pellets), powders, flakes, solids and liquids. Thus, during the transfer operation from the manufacturing process to packaging there is a potential for inhalation exposure (nuisance dust is the primary route of worker exposure) and dermal contact to liquid forms. There should be little, if any, consumer exposure to substituted p-phenylenediamines since these materials will be part of finished articles, and as such unavailable for exposure or release under typical conditions of use.

Development of the Substituted p-Phenylenediamines Category

EPA has described a stepwise process for developing categories. These steps include:

- Grouping a series of like chemicals, including the definition of criteria for the group.
- Gathering data on physicochemical properties, environmental fate and effects, and health effects for each member of the category.
- Evaluating the data for adequacy.
- Constructing a matrix of available and unavailable data.
- Determining whether there is a correlation among category members and data gathered.

Definition of the Substituted p-Phenylenediamines Category

As defined by EPA under the HPV Program, a chemical category is "a group of chemicals whose physicochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity." The similarities should be based on a common functional group, common precursors or

breakdown products (resulting in structurally similar chemicals) and an incremental and constant change across the category. The goal of developing a chemical category is to use interpolation and/or extrapolation to assess chemicals rather than conducting additional testing with specific consideration of animal welfare concerns to minimize the use of animals in the testing of chemicals.

The materials within the Substituted p-Phenylenediamines category, for the purposes of the HPV Program, are defined as phenylenediamines with alkyl, aryl or mixed alkyl-aryl substitutions, as illustrated in Figure 1.

The category referred to as Substituted p-Phenylenediamines is further categorized into two secondary subcategories; Alkylated N-PPD and 4-Aminodiphenylamine derivatives. The Alkylated N-PPD materials are structurally similar in that both N groups are alkylated, while the 4-Aminodiphenylamine Derivatives materials all contain aryl and alkyl substituted groups. Chemical structures for these materials are provided in Figure 2. The very low water solubility, low vapor pressure, slow biodegradation, low bioaccumulation potential, rapid hydrolysis and photodegradation are similar for the Substituted p-Phenylene Diamines (see Tables 1 and 3). These highly-colored, staining compounds also exhibit high flash points (see Table 1).

Matrix of SIDS Endpoints

In order to construct a matrix of SIDS endpoints for the members of the Substituted p-Phenylenediamines category, the data on physicochemical properties, environmental fate and effects, and health effects for each member of the category must be collected and evaluated for adequacy. The results of these activities are presented in the tables and text below, providing a matrix of available data for the Substituted p-Phenylenediamines materials.

Correlation within the Substituted p-Phenylenediamines Category

The matrix data patterns for physicochemical properties; environmental fate, ecotoxicity; and health effects have been evaluated for the members of the Substituted p-Phenylenediamines category. A description of the results of this evaluation follows.

Correlation of Physicochemical Properties

The physicochemical properties of the members of the Substituted p-Phenylenediamines category are presented in Table 2. These materials may exist as viscous liquids or solids at room temperature, such that melting point or boiling point data may be relevant for varying members of the category. The similarities in the other physicochemical properties of these materials, which are described below, are explained by similarities in their chemical structure, and provide justification of this group of chemicals as a category within the HPV Challenge Program.

The members of this category have a wide range of melting points and boiling points (varying based on the physical state as a liquid or solid). Six members of this category have very low vapor pressures, as indicated in Table 2. Data for six members of this category clearly indicate a lack of water solubility or negligible water solubility. Partition coefficient data are primarily in the range of 3 to 5.

Bridging to other members of the category or use of EPIWIN modeling will be used to fill physicochemical properties data requirements for the purposes of the HPV Program, as illustrated below, and in Table 1.

Alkylated N-PPD: Sufficient data exist for the Alkylated N-PPD materials for the purposes of the HPV Program.

4-Aminodiphenylamine Derivatives: Physicochemical properties data (boiling point and vapor pressure) for p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8) are provided by EPIWIN modeling. Vapor pressure, boiling point and water solubility data will be bridged from p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) to 1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4). Partition coefficient data for p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) will be bridged to p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3). EPIWIN was used to provide melting point and vapor pressure data for p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3).

Correlation of Environmental Fate

The members of this category are generally found to be not readily biodegradable by CO_2 generation, but photodegradation is rapid, as is hydrolysis. Analytical studies of hydrolysis products indicate that the molecule cleaves at the aromatic carbon-nitrogen bond.

The HPV Challenge Program requires that hydrolysis, photodegradation, biodegradation and environmental transport information be presented for each material or bridged to each member of a category. Adequate biodegradation data exist for several of the materials in this category for the purposes of the HPV Program; bridging will be used to fill the remaining biodegradation data requirements as illustrated below. The results presented indicate that these materials are poorly biodegradable, with the exception of p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4) and p-Phenylenediamine, N-(1,3-diemthylbutyl) N'-phenyl-, (793-24-8). Hydrolysis data exists for several members of this group, and gas chromatography identification and quantification of hydrolysis products suggests a common breakdown mechanism exists. Photodegradation studies presented for several members of this category are adequate for the purposes of the HPV Program; bridging will be used to fill the remaining photodegradation data requirements as illustrated below. Finally, fugacity modeling has been conducted on six of the seven members of this category, with consistent results showing partitioning to soil and/or sediment. This finding is consistent with the lack of water solubility and low vapor pressure of these materials. Bridging to other members of the category will fill environmental transport data requirements, as illustrated below.

Alkylated N-PPD: The hydrolysis data for p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9) will be bridged to p-Phenylenediamine, N,N-di-sec-butyl (101-96-2). Biodegradation and photodegradation data for p-Phenylenediamine, N,N-di-sec-butyl (101-96-2) was modeled using EPIWIN.

4-Aminodiphenylamine Derivatives: Photodegradation, hydrolysis, and environmental transport data will be bridged from p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8) to 1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4). Photodegradation data was modeled using EPIWIN for p-

Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4), p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8), p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4 and p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3).

Biodegradation data for p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3) was modeled using EPIWIN.

Correlation of Ecotoxicity

The HPV Challenge Program requires that an acute aquatic ecotoxicity test in fish, invertebrates, and algae be performed or bridged to each member of a category. Existing data (Table 4) indicate that six members of the Substituted p-Phenylenediamines category have low water solubility. The low water solubility suggests that the acute aquatic toxicity of these materials should be low due to limited bioavailability to aquatic organisms. However, the Substituted p-Phenylenediamines, in general, are very toxic to aquatic organisms. Additional testing is not necessary for these materials for the purposes of the HPV Program.

Alkylated N-PPD: Results of acute aquatic toxicity studies show p-Phenylenediamine, N, N-bis(1,4-dimethylpentyl) (3081-14-9) is harmful to algae, and very toxic to fish and Daphnia. P-Phenylenediamine, N, N-di-sec-butyl (101-96-2) was very toxic to fish and toxic to Daphnia in acute aquatic studies. The algal growth inhibition data for p-Phenylenediamine, N, N-bis(1,4-dimethylpentyl) (3081-14-9) will be bridged to p-Phenylenediamine, N, N-di-sec-butyl (101-96-2).

4-Aminodiphenylamine Derivatives: Aquatic toxicity data exist for four of the five members of this subcategory. The results of aquatic toxicity testing of these materials indicate they are toxic to very toxic to fish, Daphnia, and algae in acute studies.

The acute aquatic toxicity data for p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) will be bridged p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3).

Correlation of Health Effects

Acute Mammalian Toxicity

Acute oral and dermal toxicity data for the Substituted p-Phenylenediamines category are summarized in Table 5. The two materials in the Alkylated N-PPD subcategory of the Substituted p-Phenylene Diamines show a moderate order of acute oral toxicity. The second subcategory, the 4-Aminodiphenylamine derivatives, all have a very low order of toxicity, with LD50 values greater than the limit test of 2000 mg/kg with the exception of p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4), with an oral LD50 of 900 mg/kg. Acute dermal toxicity data for all members of the Substituted p-Phenylenediamines category demonstrate a very low order of toxicity with the dermal LD50 values greater than the limit test of 2000 mg/kg.

Adequate acute toxicity studies have been conducted for the Substituted p-Phenylenediamines category. These studies involved at least two routes of exposure (oral and dermal); and evaluated the toxicity of all the members of the category. The data demonstrate a moderate to very low order of acute toxicity. The trend in acute oral

toxicity follows the molecular weight of the materials. That is, there is a general trend toward decreasing acute oral toxicity with increasing molecular weight. The similarity in the order of toxicity for these materials is consistent with their similar chemical structure and physicochemical properties and supports the scientific justification of these materials as a category within the HPV Challenge Program.

The HPV Challenge Program requires that either an acute test be performed or bridged to each member of a category. Adequate acute oral and dermal toxicity tests exist for the Substituted N-Phenylenediamines for the purposes of the HPV Program.

Mutagenicity

A summary of the mutagenicity information for the Substituted p-Phenylenediamines category is presented in Table 6. The weight of evidence for the members of this category indicates these materials are not mutagenic.

Adequate bacterial mutagenicity tests have been conducted for all seven of the Substituted N-Phenylene diamines category to satisfy HPV Challenge requirements. Similarly, adequate *in vitro* chromosome aberration tests or *in vivo* micronucleus tests have been conducted for five of the seven materials in the Substituted N-Phenylenediamines category; additional *in vitro* or *in vivo* mammalian mutagenicity studies are available as supporting information; bridging will be used to fill the remaining data requirement.

Bacterial Gene Mutation Assay

With one exception, mutagenicity was not exhibited by any of the materials in the Substituted p-Phenylenediamines category in the bacterial mutagenicity tests with or without metabolic activation. The single exception was a positive response with 1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4).

In vivo Chromosomal Aberration Assays (Mammalian Micronucleus Test)

Three of the seven Substituted p-Phenylenediamine materials have been adequately tested in an *in vivo* chromosomal aberration assay for HPV Challenge requirements. The results were negative for clastogenicity.

In vitro Chromosomal Aberration Assay

Six of the seven Substituted p-Phenylenediamine materials have been adequately tested in an *in vitro* chromosomal aberration assay using Chinese hamster ovary cells to satisfy Program requirements. The results of these studies, performed with and without metabolic activation of the test material, were negative for clastogenicity with the exception of p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4).

The Substituted p-Phenylenediamines category has been adequately tested for mutagenicity in tests for gene mutations and chromosomal aberrations for purposes of meeting HPV Challenge requirements. The assays included point mutations in bacterial cells, *in vitro* chromosomal aberrations in mammalian cells, and *in vivo* chromosomal aberrations. The data consistently demonstrate no evidence of genotoxicity for this category of materials. 1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4) was positive in the bacterial mutagenicity test, but was negative in both in vitro and in vivo mammalian mutagenicity studies. p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) was positive for clastogenicity in the *in vitro* chromosome aberration test, but was negative in the *in vivo* mouse micronucleus test. This suggests that all

members of the category lack genotoxicity due to their similarity in chemical structures and physicochemical properties. The similarity of results for genotoxicity supports treatment of these materials as a chemical category within the HPV Challenge Program.

The HPV Challenge Program requires that a gene mutation and a chromosomal aberration test be performed or bridged to each member of a category. Bridging will be used to fill the remaining data requirements.

Alkylated N-PPD: Sufficient data exist for the Alkylated N-PPD materials for the purposes of the HPV Program.

4-Aminodiphenylamine Derivatives: Data from *in vivo* mutagenicity testing with p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8) will be bridged to p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4). Mutagenicity test data from p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) will be bridged to p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3).

By bridging these data, the category has been evaluated adequately for genotoxicity for the purposes of the HPV Program, and no additional testing is proposed**.**

Repeat Dose Toxicity

A summary of the repeat dose toxicity data for the Substituted p-Phenylene Diamines category is presented in Table 7.

Alkylated N-PPD: Adequate repeat dose studies are available for both the Alkylated N-PPD materials for the purposes of the HPV Program. P-Phenylenediamine, N, N-bis (1,4-dimethylpentyl) (3081-14-9) was given in the diet to rats at levels of 0, 100, 300, 500, 1000, or 2000 ppm (5/sex/group) for four weeks. Males at 300 ppm and above and females at 1000 ppm and above showed a reduced body weight gain. Alterations in hematology and clinical chemistry parameters were noted at the two highest dose levels. The No Observed Effect Level (NOEL) for males and females was 100 and 300 ppm, respectively. 100 male and female rats (10/sex/dose level) were dosed with p-Phenylenediamine, N, N-di-sec-butyl (101-96-2) in corn oil vehicle at 0, 10, 25, 50, or 100 mg/kg for a period of 28 days. Because the results of this study demonstrated hepatic effects in both sexes and at all treatment levels, a NOEL could not be established.

4-Aminodiphenylamine Derivatives: Adequate repeat dose studies are available for four of the five 4-Aminodiphenylamine Derivatives materials for the purposes of the HPV Program.

Subchronic studies have been conducted with p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4). When administered to rats in the diet at levels of 0, 500, 1000, 1750 and 2500 ppm for four weeks, decreases in body weight gains, hematological effects, elevations in total serum protein and increased lever and spleen weight were noted at 1000 ppm and above. The NOEL was identified as 500 ppm. In a 90-day study, p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4) was administered to rats in the diet at levels of 0, 180, 360 or 720 ppm. Lower body weight gains were observed in high-dose males; increased absolute and relative liver weights were noted in mid- and high-dose males and all treated females. Increased spleen and kidney weights were observed in high-dose females, and mild anemia was noted in mid- and high-dose animals. There

were no treatment related gross or histopathological changes noted in any group. A NOEL for organ weight changes was not established for females, while a NOEL for males was 180 ppm.

Dietary administration of p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) at 0, 500, 750, 1500 or 3000 ppm to rats for one month resulted in reduced food consumption and decreased weight gain at the three highest doses in both sexes. No gross pathology or other signs of toxicity were noted. The NOEL was identified as 500 ppm in the diet.

Dietary administration of 1,4-Benzenediamine, -mixed Ph and toyl derivatives (68953-84-4) at concentrations of 0, 120, 470 and 1900 ppm (0, 7.5, 30 and 120 mg/kg/day) to rats for 28 days resulted in body weight decreases in high dose female rats and decreased food consumption in high-dose males and mid- and high-dose females. Hematological changes (high dose), liver and kidney weight increases (high-dose male and female, mid-dose females). The No Observed Adverse Effects Level (NOAEL) for this study was established at 7.5 mg/kg. A 21-day gavage range-finding study was also conducted with rats with this material at doses of 0, 0.1, 0.3, 1 and 3 g/kg/day. Lethality was observed at 1 and 3 g/kg/day. Body weight gain loss, liver weight increase and hepatocellular labeling index increase were noted at 0.3 and/or 0.1 g/kg/day.

Santoflex 13 (p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)) was administered in feed to groups of 6 week old male and female rats at 0, 250, 1000 or 2500 ppm. Analyses via GC verified feeding levels of 0, 230, 950 and 2300 ppm. All animals survived the length of the study. Signs of toxicity during the study were limited to reduced feed consumption/body weight gain in the high-dose males and females and mid-level males. Anemia, lymphocytopenia and thrombocytosis were present in males and females, primarily at the two highest dose levels. Increases in total bilirubin in males, and total protein, albumin, globulin, calcium and/or cholesterol in both sexes were noted in high and some mid-dose level animals. Increased liver weights were observed at the two highest dose levels. There were no gross or microscopic lesions attributed to consumption of the test material. Females at low dose levels exhibited mild anemia at the interim sampling period, but all recovered by the end of the study. Therefore, the NOEL was considered to be 250 ppm.

Repeat dose data from p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) will be bridged to p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3).

By bridging these data, the category has been evaluated adequately for genotoxicity for the purposes of the HPV Program, and therefore, no additional testing is proposed**.**

Reproductive and Developmental Toxicity A summary of the reproductive and developmental toxicity data for the Substituted p-Phenylenediamines category is presented in Table 7.

Alkylated N-PPD: Adequate reproductive toxicity studies are available for the purposes of the HPV Program for one of the two Alkylated N-PPD materials. P-Phenylenediamine, N, N-bis (1,4-dimethylpentyl) (3081-14-9) was not embryotoxic, fetotoxic or teratogenic when administered by gavage at doses of 0, 25, 75 or 150 mg/kg/day to pregnant rats on gestation days 6-15. Administration of CAS No. 3081-14-9 at dietary concentrations of 0, 30, 100 or 300 ppm to male and female rats for three successive generations produced no adverse effects on mating or fertility indices. Reduced survival of offspring was observed in mid- and high-dose

groups; however, evidence of parental toxicity was also present as indicated by reduced body weight gains of mid- and high-dose groups. The NOEL was 30 ppm. The developmental and reproductive studies with p-Phenylenediamine, N, N-bis (1,4-dimethylpentyl) (3081-14-9 will be bridged to p-Phenylenediamine, N, N-di-sec-butyl (101-96-2).

4-Aminodiphenylamine Derivatives: Adequate reproductive and developmental toxicity studies are available for three of the five 4-Aminodiphenylamine Derivatives materials for the purposes of the HPV Program.

p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4) was administered to rats by gavage at dose levels of 0, 12.5, 62.5 or 125 mg/kg/day for gestation days 6-15. The NOEL for maternal toxicity was determined to be 62.5 mg/kg. There were significant skeletal effects at 125 mg/kg and the NOEL for teratogenicity was established at 62.5 mg/kg.

1,4-Benzenediamine, N, N'-mixed Ph and toyl derivatives (68953-84-4) was administered in feed at 0, 120, 400 or 1500 ppm to rats in a two-generation reproductive toxicity study. Dystocia (potentially leading to prolonged gestation and increased perinatal deaths, decreased live births and increased pup weights), and polycystic lesions were observed at all dose levels; a NOAEL was not established in this study. A developmental study was also conducted with 1,4-Benzenediamine, N, N'-mixed Ph and toyl derivatives (68953-84-4) in rats. The test article was administered by gavage at dose levels of 0, 20, 70 and 200 mg/kg/day for gestation days 6-15. The test article produced minimal effects (body weight) to maternal rats at 200 mg/kg during pregnancy; the NOAEL for maternal toxicity was established at 70 mg/kg/day. There were no birth defects observed in fetal animals and the NOAEL for teratogenicity/developmental effects was established at 200 mg/kg/day.

A reproductive oral gavage study was conducted in rats with p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8); no reproductive effects were observed at the highest concentration tested (1000 ppm). In a rat gavage developmental study, the test article was administered by gavage at dose levels of 0, 50, 100 and 250 mg/kg/day for gestation days 6-15. A NOAEL (teratogenicity /developmental effects) greater than 250 mg/kg/day was determined. The NOEL for maternal toxicity was established at 50 mg/kg/day.

Data from these three studies materials will be bridged to p-Phenylenediamine, N- (1,4-dimethylpentyl) N'-phenyl (3081-01-4) and p-Phenylenediamine, N, (1-methylheptyl)-N'-phenyl, (15233-47-3).

Test Plan

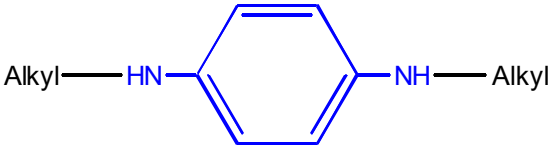
Table 8 provides the category test plan for the Substituted p-Phenylenediamines. All HPV endpoint requirements are fulfilled by existing adequate data, calculated data, or by bridging data based on SAR and the category approach. The chemicals that constitute the Substituted p-Phenylenediamines category require no additional testing for the purposes of the HPV Program.

FIGURES

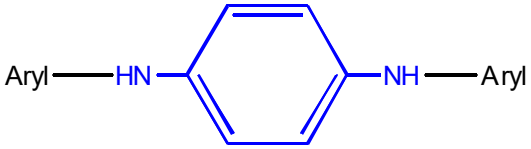
Figure 1. Structural Definition

Phenylenediamine with various aryl or alkyl substitutions in the para position:

Alkyl-N-Phenyl-N-Alkyl (all Alkyl)
Aryl-N-Phenyl-N-Aryl (All Aryl)
Alkyl-N-Phenyl-N-Aryl (Mixed Alkyl-Aryl)



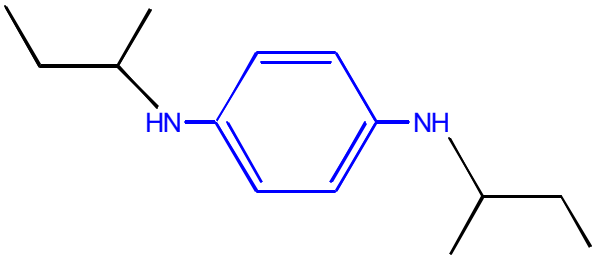
Alkyl-Alkyl Substitutions



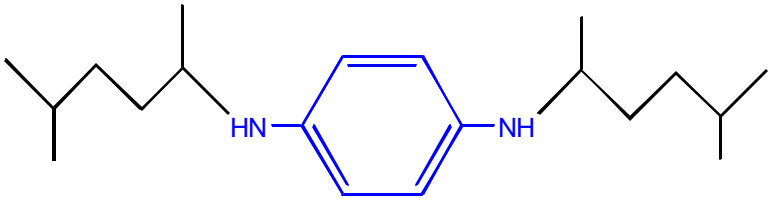
Aryl-Aryl Substitutions

Mixed Alkyl-Aryl Substitutions

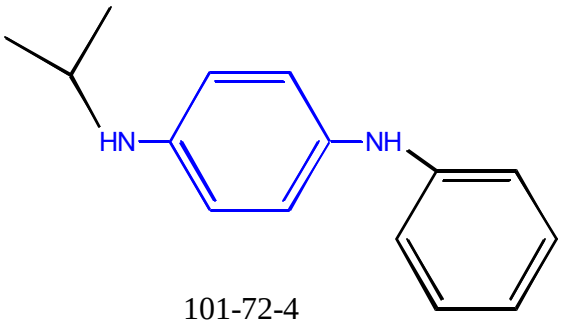
FIGURE 2. Chemical Structures



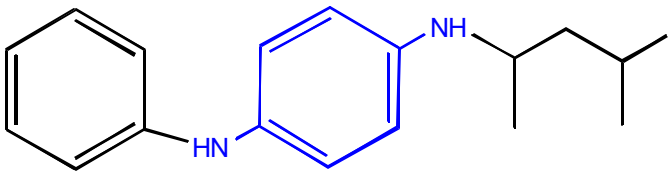
101-96-2
Alkyl-Alkyl



3081-14-9
Alkyl-Alkyl

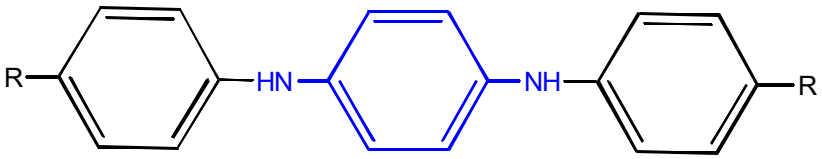


101-72-4
Alkyl-Aryl

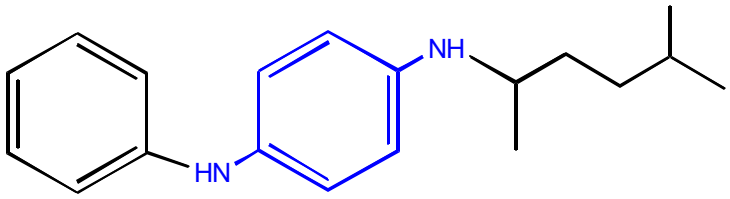


793-24-8
Alkyl-Aryl

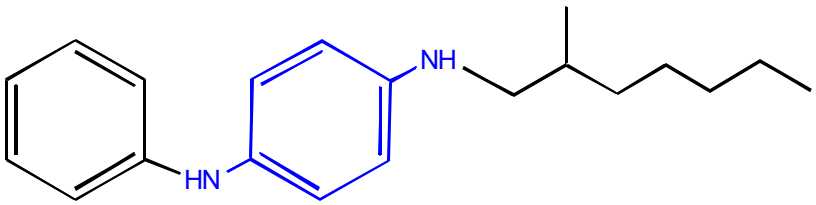
R = H or CH3



68953-84-4
Aryl-Aryl (Mixed)



3081-01-4
Alkyl-Aryl



15233-47-3
Alkyl-Aryl

TABLES

Table 1. Justification of the Substituted p-Phenylenediamines Category using Flash Point, Vapor Pressure, Water Solubility and Biodegradation

Name (CAS No.)/ Molecular weight	Flash Point (^{o}F)	Vapor Pressure (mm Hg @ 20^{o}C)	Water Solubility	Bio-degradability
Alkylated N-PPD				
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)/ 220.4	290	85.3 @ 33C	Insoluble	Not readily biodegradable
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)/ 304	182	1.1 @ 25C	Very Slight	Not readily biodegradable
4-Aminodiphenylamine derivatives				
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)/ 226.4	>200 C	3.4E-5 @ 90C	Insoluble	Readily biodegradable
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)/ 268.5	400	4.93E-6 @ 25C (EPIWIN)	Insoluble	Readily biodegradable
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)/ 274	450	Not determined	Not determined	Not readily biodegradable
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)/ 282	420	1.25E-10 @25C	Insoluble	Not readily biodegradable
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3)/ 296	Not determined	4.99E-7 @ 25C (EPIWIN)	Insoluble	Not readily biodegradable

Table 2. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members Physicochemical Properties

Name (CAS No.)	Melting Point (°C)	Vapor Pressure (mm Hg @ 20°C)	Boiling Point (°C)	Partition Coefficient	Water Solubility
Alkylated N-PPD					
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	18	85.3 @ 38 C	98 @ 26.6hPa	3.50	<1 mg/ml @ 20C
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	-36	<1.1E-6 @ 25C	183.5 @ 1mm Hg	5.34	21 ppm @ pH5; 0.8 ppm @ pH 9
4-Aminodiphenylamine derivatives					
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	75-80	3.4E-3 @90C	161	3.28	15 ppm @25C
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	45	4.93E-6 @25C (EPIWIN)	369.67 (EPIWIN)	4.7	1 ppm @ 23C
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	90-105	Not determined	Not determined	3.4-4.3	Not determined
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	32	1.25E-10 @ 25C	231 @3.5 mmHg	5.17	0.67g/l @ 25C
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3)	145.77 (EPIWIN)	4.99E-7 @ 25C (EPIWIN)	431	Not determined	Insoluble

Table 3. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members Environmental Fate

Name (CAS No.)	Hydrolysis	Photo-degradation (t1/2 in hours)	Bio-degradation	Environmental Transport
Alkylated N-PPD				
p-Phenylenediamine, N, N-di-sec-butyl (101-96-2)	Not determined	1.095 (EPIWIN)	Not readily biodegradable (EPIWIN)	Primarily to soil
p-Phenylenediamine, N, N-bis(1,4-dimethylpentyl) (3081-14-9)	97%@pH7 after 24 hr	2	50% after 35 days	Primarily to sediment
4-Aminodiphenylamine derivatives				
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	99%@pH7 after 24 hr	0.588 (EPIWIN)	98% after 22 hours	Primarily to soil
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	93%@pH7 after 24 hr	0.567 (EPIWIN)	50 % after 2.9 hours	Primarily to soil
1,4-Benzenediamine, N, N'-mixed Ph and toyl derivatives (68953-84-4)	Not determined	Not determined	0.64% after 28 days	Not determined
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	96%@pH7 after 24 hr	0.563 (EPIWIN)	0% @ 35days	Primarily to soil
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	Not determined	0.56 (EPIWIN)	Not readily biodegradable (EPIWIN)	Primarily to soil and sediment

Table 4. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members Ecotoxicity

Name (CAS No.)	Acute Fish 96-hour LC50 (mg/l)	Acute Invertebrate 48-hour EC50 (mg/l)	Algal growth inhibition 96-hour EC50 (mg/l)
Alkylated N-PPD			
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	0.13	1.4	Not determined
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	0.28	0.37	52
4-Aminodiphenylamine derivatives			
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	0.34	1.1	0.5 (cell growth)
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	0.14-0.4	0.82	0.6
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	0.48	1.8	(72-hour EC50) 0.018 (biomass); >0.079 (growth rate)
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	0.3-1.1	0.2	0.7
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	Not determined	Not determined	Not determined

Table 5. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members
Acute Toxicity

Name (CAS No.)	Acute Oral (mg/kg)	Acute Dermal (mg/kg)
Alkylated N-PPD		
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	271	2806
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	730	>3160
4-Aminodiphenylamine derivatives		
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	900	>7940
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	>5000	>7940
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	>2000	>2000
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	>2000	>5010
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3)	4300	>2000

Table 6. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members Genotoxicity

Name (CAS No.)	Genotoxicity (*in vitro* - bacterial)	Genotoxicity (*in vitro* - mammalian)	Genotoxicity (*in vivo*)
Alkylated N-PPD			
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	Negative	Negative	Not determined
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	Negative	Negative	Not determined
4-Aminodiphenylamine derivatives			
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	Negative	Negative	Not determined
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	Negative	Negative	Negative
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	Positive	Negative	Negative
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	Negative	Weak Positive; Supporting data Negative	Negative
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	Negative	Not determined	Not determined

Table 7. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members Health Effects

Name (CAS No.)	Repeat Dose	Reproductive	Developmental
Alkylated N-PPD			
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	28-Day oral gavage with rats. NOEL< 10 mg/kg/day	Not determined	Not determined
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	30 day feeding study with rats. NOEL (males) 100 pm; (females) 300 ppm	Three generation rat oral feeding study; NOEL(parental, F1 and F2 offspring) = 30 ppm	Rat gavage: NOEL (teratogenicity) = >150 mg/kg/day; (maternal) = 25 mg/kg/day
4-Aminodiphenylamine derivatives			
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	90-day feeding study with rats. NOEL (males) 180 ppm; NOEL not established (females)	Not determined	Rat gavage: NOEL (teratogenicity) = 62.5, (maternal) 62.5 mg/kg/day
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	90-day oral rat-NOAEL = 250 ppm in feed	Rat gavage – NOEL (parental) >1000 ppm; (F1 offspring) >1000 ppm	Rat gavage: NOAEL (teratogenicity /developmental effects) = 250 mg/kg/day; NOEL (maternal) = 50 mg/kg/day
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	28-day rat oral NOAEL = 7.5 mg/kg	Two generation rat oral feeding study – NOEL not identified	Rat gavage: NOAEL (teratogenicity /developmental effects) $\leq$ 200 mg/kg/day, NOAEL (maternal toxicity 70 mg/kg/day)
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	1 month feeding study with rats – NOEL = 500 ppm in diet	Not determined	Not determined
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	Not determined	Not determined	Not determined

.

Table 8

Substituted p-Phenylenediamines Category Test Plan

CAS Nos. 101-96-2, 3081-14-9, 101-72-4, 793-24-8, 3081-01-4, 15233-47-3, and 68953-84-4
Rubber and Plastic Additives Panel American Chemistry Council
December 2001

	Physical-Chemical				
CHEMICAL	**Melting Point**	**Boiling Point**	**Vapor Pressure**	**Partition Coefficient**	**Water Solubility**
Alkylated N-PPD					
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	A	A	A	A	A
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	A	A	A	A	A
4-Aminodiphenylamine derivatives					
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	A	A	A	A	A
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	A	Calc	Calc	A	A
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	A	R	R	A	R
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	A	A	A	A	A
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	R	A	Calc	R	A

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

Table 8 (continued)

CHEMICAL	Environmental Fate			
	Photo-degradation	Hydrolysis	Environmental Transport	Biodegradation
Alkylated N-PPD				
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	Calc	R	Calc	Calc
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	A	A	Calc	A
4-Aminodiphenylamine derivatives				
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	Calc	A	Calc	A
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	Calc	A	Calc	A
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	R	R	R	A
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	Calc	A	Calc	A
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	Calc	R	Calc	Calc

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

Table 8 (continued)

CHEMICAL	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Plants (e.g., Algae)	Acute Toxicity to Aquatic Invertebrates (e.g., Daphnia)
Alkylated N-PPD			
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	A	R	A
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	A	A	A
4-Aminodiphenylamine derivatives			
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	A	A	A
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	A	A	A
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	A	A	A
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	A	A	A
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	R	R	R

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

Table 8 (continued)

CHEMICAL	Mammalian Toxicity						
	Acute Toxicity	Genetic Toxicity *In Vitro (bacterial)*	Genetic Toxicity *In Vitro (mammalian)*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
Alkylated N-PPD							
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	A	A	A	NR	A	R	R
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	A	A	A	NR	A	A	A
4-Aminodiphenylamine derivatives							
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	A	A	A	R	A	R	A
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	A	A	A	A	A	A	A
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	A	A	A	A	A	A	A
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	A	A	R	A	A	R	R
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	A	A	R	R	R	R	R

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

AR201-13383B

101-96-2
p-Phenylenediamine, N,N'-di-sec-Butyl

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	18°C
Decomposition:	Yes [ ] No [X] Ambiguous [ ]
Sublimation:	Yes [ ] No [X] Ambiguous [ ]
Method:	Not determined
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	HSDB, NTP Chemical Repository
Reference:	Ashford's Dictionary of Industrial Chemicals, 1994

*2.2 BOILING POINT

Value:	98°C
Pressure:	at 26.6 hPa
Decomposition:	Yes [ ] No [X] Ambiguous [ ]
Method:	Not determined
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	HSDB
Reference:	Kirk-Othmer Encyclopedia of Chemical Technology, 1991

†2.3 DENSITY (relative density)

Type:	Bulk density [X]; Density []; Relative Density [ ]
Value:	0.94 kg/l
Temperature:	20°C
Method:	Not Determined
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	HSDB
Reference:	Ashford's Dictionary of Industrial Chemicals, 1994

*2.4 VAPOUR PRESSURE

Value:	85.3 mm Hg
Temperature:	38°C
Method:	calculated [ ]; measured [X] Instrumental method
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	Radian Research
Reference:	NTP Chemical Repository, 2001

*2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

Log Pow:	3.50
Temperature:	Not determined
Method:	calculated [X]; measured [ ] SRC LogKow (KowWin) Program 1995
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	
Reference:	Meylan, W.M. and. P.H. Howard, 1995 J. Pharm. Sci. 84: 83-92

*2.6 WATER SOLUBILITY

A. Solubility

Value:	<1 mg/ml
Temperature:	20°C
Description:	Miscible **[ ]**; Of very high solubility **[ ]**; Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble **[ ]**; Of low solubility **[ ]**; Of very low solubility **[ ]**; Not soluble **[X]**
Method:	Not determined
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	Radian Research
Reference:	NTP Chemical Repository, 2001

B. pH Value, pKa Value

pH Value:	. . . . .
Concentration:	. . . . .
Temperature:	. . . . . °C
Method:.	
GLP:	Yes **[ ]** No **[ ]** ? **[ ]**
pKa value	. . . . . at 25°C
Remarks:	
Reference:	

2.11 OXIDISING PROPERTIES

Results:	Maximum burning rate equal or higher than reference mixture**[]**; Vigorous reaction in preliminary test **[ ]**; No oxidising properties **[ ]**; Other **[ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[ ]**
Remarks:	
Reference:	

†2.12 OXIDATION: REDUCTION POTENTIAL

Value:	. . . . . mV
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[ ]**
Remarks:	
Reference:	

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

Value:	. . . . .
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[ ]**
Remarks:	
Reference:	

B. Other data

Results:	
Remarks:	
Reference:	

3. <u>ENVIRONMENTAL FATE AND PATHWAYS</u>

***3.1.1 PHOTODEGRADATION**

Type: Air **[X]**; Water **[ ]**; Soil **[ ]**; Other **[ ]**
Light source: Sunlight **[ ]**; Xenon lamp **[ ]**; Other **[ ]**
Light spectrum: . . . . . nm
Relative intensity: . . . . . *(based on intensity of sunlight)*
Spectrum of substance: . . . . . nm
Concentration of Substance: . . . . . .
Temperature: . . . . . °C
Direct photolysis:
Half life: . . . . . .
Degradation: . . . . . % (weight/weight) after . . . . . (exposure time)
Quantum yield: . . . . . .
Indirect Photolysis:
Type of sensitizer: . . . . . OH . .
Concentration of sensitizer: . 1560000 . . . molecule/cm^3. . . . . .
Rate constant (radical): . . . 117.2377 E-12 .. cm^3/ molecule *sec
Degradation: . . . . . 50% at 1.095 Hrs. . . .
Method: calculated **[X]**; AOP Program (v1.89)
measured **[ ]**

GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: . molecular structure, purity:. . . . . .
Remarks:
Reliability: (2) valid with restrictions
Accepted calculation method
Reference: Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

***3.1.2 STABILITY IN WATER**

Type: Abiotic (hydrolysis) **[ ]**; biotic (sediment)**[ ]**
Half life: . . . . . at pH . . . . . at . . . . . °C
Degradation: . . . . . at pH . . . . . at . . . . . °C after . . . . . (exposure time)
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance: . . . . . , purity: . . . . . .
Remarks:
Reference:

***3.2 MONITORING DATA (ENVIRONMENTAL)**

Type of Measurement: Background **[ ]**; At contaminated site **[ ]**; Other **[ ]**
Media:
Results:
Remarks:
Reference:

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION

3.4

***3.3.1 TRANSPORT**

Type: Adsorption **[ ]**; Desorption **[ ]**; Volatility **[ ]**; Other **[ ]**
Media:
Method:
Results:
Remarks:
Reference:

***3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)**

Media: Air-biota **[ ]**; Air-biota-sediment-soil-water **[ ]**; Soil-biota **[ ]**; Water-air **[ ]**; Water-biota **[ ]**; Water-soil **[ ]**; Other **[ ]**

Method: Fugacity level I **[ ]**; Fugacity level II **[ ]**; Fugacity level III **[X]**; Fugacity level IV**[]**;Other (calculation)**[]**;Other (measurement)**[ ]**

Results:

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	0.0952	2.19	1000	2.37e-012
Water	26.1	900	1000	2.36e-013
Soil	72.6	900	1000	2.33e-013
Sediment	1.24	3.6e+003	0	1.75e-013

	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	678	21.4	22.6	0.714
Water	451	586	15	19.5
Soil	1.26e+003	0	41.9	0
Sediment	5.35	0.556	0.178	0.0185

Persistence Time: 750 hr
Reaction Time: 940 hr
Advection Time: 3.7e+003 hr
Percent Reacted: 79.7
Percent Advected: 20.3

Remarks:
Reliability: (2) valid with restrictions
Accepted calculation method
Reference: Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

***3.5 BIODEGRADATION**

Type: aerobic **[ ]**; anaerobic **[ ]**
Inoculum: adapted **[ ]**; non-adapted **[ ]**;
Concentration of the chemical: . . . . . related to COD **[ ]**; DOC **[ ]**; test substance **[ ]**
Medium: water **[ ];** water-sediment **[ ];** soil **[ ];** sewage treatment **[ ]**
Degradation: *(percentage reduction/exposure time)*
. . . . . % after . . . . . (time)
Results: readily biodeg. **[ ];** inherently biodeg. **[ ]**; under test condition no biodegradation observed **[ ]**, other **[ ]**
Kinetic . . . . . % in . . . . . (time)
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance: . . . . . , purity: . . . . . .

Remarks:
Reference:

4. ECOTOXICITY

*4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]**
open-system **[ ]**; closed-system **[X]**
Species: Salmo gairdneri (Rainbow Trout)
Exposure period: 96 Hours
Results: LC_{50} (24h) = >0.18 mg/l
LC_{50} (48h) = 0.14 mg/l
LC_{50} (72h) = Not determined
LC_{50} (96h) = 0.13 mg/l
NOEC = 0.056 mg/l
LOEC = 0.10 mg/l
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:. Santoflex 44 dark red liquid #KB12-902, purity: >97%
Remarks: Acetone used as solvent. Range-finding experiment conducted to determine final bioassay test concentrations. Water temperature, dissolved oxygen content, and pH monitored throughout study. Quality check via challenge with reference compound Antimycin A. Data reported at 95% confidence level.
Reference: Monsanto AB-83X-036, Analytical Bio-Chemistry Labs, 1983

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]**
open-system **[ ]**; closed-system **[X]**
Species: Lepomis machrochirus (Bluegill Sunfish)
Exposure period: 96 Hours
Results: LC_{50} (24h) = 0.19 mg/l
LC_{50} (48h) = 0.18 mg/l
LC_{50} (72h) = Not determined
LC_{50} (96h) = 0.18 mg/l
NOEC = 0.10 mg/l
LOEC = 0.18 mg/l
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:. Santoflex 44 dark red liquid #KB12-902, purity: >97%
Remarks: Acetone used as solvent. Range-finding experiment conducted to determine final bioassay test concentrations. Water temperature, dissolved oxygen content, and pH monitored throughout study. Quality check via challenge with reference compound Antimycin A. Data reported at 95% confidence level.
Reference: Monsanto AB-83X-035, Analytical Bio-Chemistry Labs, 1983

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]**
open-system **[ ]**; closed-system **[X]**
Species: Pimephales promelas (Fathead Minnows)
Exposure period: 96 Hours
Results: LC_{50} (24h) = 0.13 mg/l
LC_{50} (48h) = 0.13 mg/l
LC_{50} (72h) = Not determined
LC_{50} (96h) = 0.13 mg/l
NOEC = 0.10 mg/l
LOEC = 0.18 mg/l
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:. Santoflex 44 dark red liquid #KB12-902, purity: >97%
Remarks: Acetone used as solvent. Range-finding experiment conducted to determine final bioassay test concentrations. Water temperature, dissolved oxygen content, and pH monitored throughout study. Quality check via challenge with reference compound Antimycin A. Data reported at 95% confidence level.
Reference: Monsanto AB-84X-021, Analytical Bio-Chemistry Labs, 1983

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]**;
open-system **[ ]**; closed-system **[X]**
Species: Daphnia magna
Exposure period: 48 Hours
Results: EC_{50} (24h) = 2.0 mg/l
EC_{50} (48h) = 1.4 mg/l
NOEC = 0.56 mg/l
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex 44 dark liquid Lot# KB12-902, purity:>97%
Remarks: Acetone used as solvent. Range-finding experiment conducted to determine final bioassay test concentrations. Water temperature, dissolved oxygen content, and pH monitored throughout study. The abnormal effects of mortality and daphnids lying on the bottom progressed from 3.2 mg/l initially, to 1.0 mg/l after 48 hours.
Reference: Monsanto AB-83X-037, Analytical Bio-Chemistry Labs, 1983

*4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae

Species:
Endpoint: Biomass **[ ]**; Growth rate **[ ]**; Other **[ ]**
Exposure period:
Results: EC_{50} (. . . . . .h) = ... mg/l
EC_{xx} (. . . . . .h) = ... mg/l

NOEC = ... mg/l
LOEC = ... mg/l
Analytical monitoring: Yes **[ ]** No **[ ]** ? **[ ]**
Method:

open-system **[ ]**; closed-system **[ ]**
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance: . . . . . , purity: . . . . . .
Remarks:
Reference:

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Type: LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain: Sprague-Dawley Albino Rats
Value: 271 mg/kg bw for males and females combined
281 mg/kg for males
265 mg/kg for females
Method: Finney, J.D., Reference for Method of LD50 Determination, Probit Analysis 3rd Edition, 1971
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex 44, Lot S-40182, purity: 96.09%
Remarks: Groups of five male and five female rats were dosed by oral gavage with the test article as a 392 mg/ml solution in corn oil. Clinical observations were made 3x/day during the first 8 hours, and 2x/day thereafter until sacrifice. After a 14-day recovery period, all surviving animals were sacrificed. Necropsies were performed on all animals. Clinical signs of toxicity included lethargy, ataxia, ptosis, and abnormal urine coloration (green and/or reddish-brown). Necropsy findings included gastrointestinal inflammation, which reached the severity of hemmorhage in many cases, gastrointestinal distension, and red, fluid-filled gastric masses. The presence of these masses indicated that the toxicity to gastrointestinal tissue may have contributed to lethality in virtually all rats that died during the test. Previous oral and dermal toxicity studies with this material have noted the corrosivity to tissue that complicates accurate determinations of LD50 values.
Reference: Monsanto ML-82-181, Environmental Health Labs, 1983

5.1.2 ACUTE INHALATION TOXICITY

Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[ ]**; LCL_0 **[X]**; Other **[ ]**
Species/strain: Sprague-Dawley Albino Rats
Exposure time: 6 Hours
Value: 600 mg/m3
Method: Not Determined
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance:. Cas # 101-96-2, purity: Commercial (>96%)
Remarks: RTECS and NTP reference. Test conditions unknown.
Reference: Kodak Company Reports, 1971

Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[X]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain: Sprague-Dawley Albino Male Rats
Exposure time: 6 Hours
Value: >0.2 mg/l
Method: A.T.S. 8/1973
GLP: Yes **[ ]** No **[X]** ? **[ ]** **Klimisch 2**
Test substance:. Santoflex 44 Lot# 24277, purity: >96%
Remarks: Six male rats were exposed to the test article at a concentration of 0.2 mg/l at ambient temperature at an airflow rate of 4 l/min for six hours. The difference in weight of the sample after the test indicated that 0.4 grams had been vaporized under test conditions. There were no clinical signs of toxicity noted during the experiment. Following a 14-day recovery period, all animals were sacrificed. Necropsy findings were that all viscera examined appeared normal.
Reference: Monsanto Y-76-262, Younger Laboratories, 1976

5.1.3 ACUTE DERMAL TOXICITY

Type: LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain: New Zealand Albino Rabbits
Value: 2806 mg/kg bw
Method: Finney, J.D., Reference for Method of LD50 Determination, Probit Analysis 3rd Edition, 1971
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex 44 Lot S-40182, purity: 96.09%
Remarks: Groups of four male and female rabbits were exposed to the test article via a single dermal application to shaved skin. Two animals from each group were predesignated to have their skin abraided in the treatment area. Skin of the other animals was intact. Clinical observations were made 3x/day during the first eight hours after exposure, then 2x/day thereafter until sacrifice. Necropsies were performed on all animals. Clinical signs of toxicity included lethargy, ataxia, green coloration of the urine, partial loss of ability to move the limbs, and localized dermal effects attributed to the direct contact between skin and test article. Findings on necropsy included green material in the bladder of sixteen animals, four animals with an enlarged gall bladder, and five with hepatic discoloration.
Reference: Monsanto ML-82-022, Environmental Health Lab, 1983

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

Species/strain: New Zealand White Rabbits
Results: Highly corrosive **[ ]**; Corrosive **[X]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[x]**; Not irritating **[ ]**
Classification: Highly corrosive (causes severe burns) **[ ]**; Corrosive (causes burns) **[X]**; Irritating **[ ]**; Not irritating **[ ]**
Method: Draize, J.H. Woodard, G., and Calvery, H.O., Methods for the Study of Irritation and Toxicity of Substances Applied Topically

To the Skin and Mucous Membranes, J. Pharmacol. Exp. Therap. 82: 377-390, 1944

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance: Santoflex 44 Lot S-40182, purity: 96.09%

Remarks: The test undiluted article, at a volume of 0.5 ml, was applied to the intact and abraided shaved skin of six rabbits for 24 hours. The initial observation was made approximately one hour after exposure. Dermal irritation was scored by the Draize Method, and results recorded on day 1, 3, 7, 10, 14 and 17 after exposure. Scarring, hardening of the skin, scabbing and sloughing skin were noted on all animals. The test article was classified as corrossive under the test conditions.

Reference: Monsanto ML-82-022c, Environmental Health Lab, 1983

SKIN IRRITATION/CORROSION

Species/strain: New Zealand Albino Rabbits

Results: Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[X]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[ ]**

Classification: Highly corrosive (causes severe burns) **[ ]**; Corrosive (causes burns) **[ ]**; Irritating **[X]**; Not irritating **[ ]**

Method: D.O.T. Hazardous Material Regulations 49 CFR 173.240, 1976

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance:. Antioxidant PDA #1549-83, purity: Not stated

Remarks: The undiluted test article was applied to the shaved skin of six rabbits in a single application of 0.5 ml. The test site was covered for four hours with surgical gauze and an elastic bandage. The entire trunk of the rabbit was wrapped in 2 mil thick plastic to prevent evaporation of the test article, and the plastic was covered with a white cotton towel. After four hours, the wrappings were removed, and the skin allowed to equilibrate for hydration and compression for 30 minutes. Skin was scored for erythema, eschar formation and corrosion in accordance with the Federal Hazardous Substances Act Grading Code, 16 CFR 1500.41. After grading, the test site was washed with water. Test sites were scored again after 24, 48 and 72 hours, and 1 and 2 weeks. Gross observations of corrosion were noted in 2/6 rabbits at I week and in 4/6 rabbits after 2 weeks. Under the conditions of the DOT test, these results were judged to be between "marginal" and "severely irritating but not corrosive". Because of the results of earlier studies, the manufacturers of this material have chosen to classify it as "corrosive" for both use and transportation.

Reference: Monsanto XX-84X-144, Gulf South Research, 1983

5.2.2 EYE IRRITATION/CORROSION

Species/strain: New Zealand Albino Rabbits

Results: Highly corrosive **[ ]**; Corrosive **[X]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[ ]**

Classification: Irritating **[]**; Not irritating **[]**; Risk of serious damage to eyes **[X]**

Method: Draize et.al., J. Pharmacol., Exp. Therap. 82: pp 377-390, 1944

GLP: Yes **[ ]** No **[ ]** ? **[x]**
Test substance:. Santoflex 44 Lot# S-40182, purity 96.09%
Remarks: A single dose of 0.1 ml of the undiluted test article was placed in the one eye of three male and three female rabbits, with the untreated eye serving as the control. A topical anesthetic available if discomfort appeared severe. Signs of irritation were scored according to the Draize procedure. Scoring will be done at 24, 48 and 72 hours after treatment. Discomfort on application was slight. Observations at 24 hours included severe erythema with necrosis, severe edema, copious discharge containing a whitish exudate and severe swelling of conjunctivae. Under the test conditions, the material was classified as "corrosive". Scabs sloughed off in 14 to 21 days with no apparent permanent corneal damage.
Reference: Monsanto ML-82-022d, Environmental Health Laboratory, 1983

***5.4 REPEATED DOSE TOXICITY**

Species/strain: Sprague-Dawley Albino Rats
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral gavage
Exposure period: 28 days
Frequency of treatment: Daily
Post exposure observation period:
Dose: 0, 10, 25, 50, or 100 mg/kg
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[]**; Concurrent vehicle **[X]**; Historical **[]**
NOEL: <10 mg/kg
LOEL: 10 mg/kg
Results: 100 male and female rats (10/sex/dose level) were dosed with the test article in corn oil vehicle at the above levels for a period of 28 days. The animals were observed 2x/day for mortality or signs of toxicity. Detailed observations, body weights and feed consumption was documented 1x/week. Hematology determinations and clinical chemistry determinations were made on all control animals and the high-dose animals prior to terminal sacrifice. Additional clinical chemistry determinations of GGTP, SGOT, Sgtp, Bilirubin, SAP and 5-nucleotidase were performed on all treated animals. A complete gross necropsy was performed on all animals at sacrifice and within 16 hours of any animal who died during the course of the study. Two mid-dose males died within the first week of treatment and two high-dose females died during week 3. Cause of death did not appear to be treatment-related. One additional mid-dose female was sacrificed at day 15 following an injury during dosing. All other animals survived to sacrifice. Gross necropsy findings on two high-dose females was a slightly pale liver. In males, a finding of dilation of the right renal pelvis was found in several animals at all dose levels, including controls. Adverse effects observed included increased liver weights and elevation of serum enzymes SGOT, Sgpt and GGTP, indicative of hepatocellular damage, as well as a dose-dependent increase in the incidence of hepatocellular lesions. Because the results of this study demonstrated hepatic effects in

both sexes and at all treatment levels, a No Observed Effect Level could not be established.

Method: OECD Guidelines for the Testing of Chemicals, 1981
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:. Santoflex 44 Lot# KC11-928, purity: >96%
Reference: Monsanto PR-83-317, Pharmacopathics Research Labs, 1984

Species/strain: Sprague-Dawley Albino Rats
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral dietary
Exposure period: 90-94 days
Frequency of treatment: Daily
Post exposure observation period:
Dose: 0, 20, 100 or 500 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[]**; Historical **[]**
NOEL: 100 mg/kg
LOEL: 500 mg/kg
Results: In a subchronic feeding study, groups of male and female rats were fed the test article via dietary admixture for three months. After 65 days of treatment, the low-dose (20 ppm) group was increased to 1000 ppm for twenty-five days, and then to 2000 ppm for the final four days of the study. Findings included decreased body weights and body weight gain in the 500 ppm males, and decreased body weights in the 500 ppm females. There were no clinical signs of toxicity noted for any dose level for either sex. All animals survived until terminal sacrifice. Hematology determinations and clinical chemistry determinations were made on all animals prior to sacrifice, and all animals received a complete gross necropsy. There were no hematological or histopathological findings at any dose level that were considered to be treatment-related. The NOEL was determined to be 100 ppm, or 6.6 mg/kg/day, for both males and females based upon the reduced body weights seen at 500 ppm.
Method: Not determined
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance:. Antioxidant 22, purity: Commercial grade, 96% minimum
Reference: E.I. DuPont de Nemours, unpublished data, 1987

*5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

Type: Bacterial Reverse Mutation - Ames
System of testing: Salmonella typhimurium TA97, TA98, TA100, TA1535, TA 1537, TA1538
Concentration: Not determined
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:
Cytotoxicity conc: With metabolic activation: Not determined
Without metabolic activation: Not determined

Precipitation conc: Not determined
Genotoxic effects: + ? -
With metabolic activation: **[ ] [ ] [X]**
Without metabolic activation: **[ ] [ ] [X]**
Method: OECD 471 Plate Overlay method
GLP: Yes **[]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance: N,N'-di-sec-butyl-p-phenylenediamine, purity: Technical grade
Remarks: The test compound was tested in Ames/Salmonella plate incorporation assays using the tester strains TA 97, TA98, T A100, TA1535, and TA1538 and TA1537 in the presence and absence of an Aroclor-induced rat liver mammalian metabolic activation system (S-9 Mix). No mutagenic activity was observed for the test compound in any of these assays.
Reference: Zeiger, et. al., Environ. Mol. Mutagen, 1998

B. NON-BACTERIAL IN VITRO TEST

Type: CHO and CHL Forward Gene Mutation Assay
System of testing: Cultured Chinese hamster ovary (CHO) cells and cultured Chinese Hamster Lung (CHL) cells
Concentration: Not determined
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:
Cytotoxicity conc: With metabolic activation: Not determined
Without metabolic activation: Not determined
Precipitation conc: Not determined
Genotoxic effects: + ? -
With metabolic activation (CHO): **[ ] [ ] [X]**
Without metabolic activation (CHO): **[ ] [ ] [X]**
With metabolic activation (CHL): **[ ] [ ] [X]**
Without metabolic activation (CHL): **[ ] [X] [ ]**
Method: OECD 476
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance:. N,N'-di-sec-butyl-p-phenylenediamine, purity: Commercial grade
Remarks: The test article was one of 25 chemicals tested for the induction of chromosomal aberrations in two cultured mammalian cell systems the cultured cells from Chinese hamster ovaries (CHO, and those from Chinese hamster lungs (CHL), in the presence absence of metabolic activation with the S9 mix. The test article negative with metabolic activation in both CHO and CHL cells, and negative without metabolic activation in CHO cells. The results for CHL cells without metabolic activation were equivocal. Overall, the results indicate that the test article is negative for the potential to cause chromosomal aberrations, both with and without metabolic activation, under the test conditions.
Reference: Sofuni, et.al. Mutation Research, 1990

*** 5.6 GENETIC TOXICITY IN VIVO**

Type:
Species/strain:
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration:
Exposure period:

Doses:
Results:
Effect on mitotic
index or P/N ratio:
Genotoxic effects: + ? -
[ ] [ ] [ ]
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance: . . . . . , purity: . . . . . .
Remarks:
Reference:

***5.8 TOXICITY TO REPRODUCTION**

Type: Fertility **[]**; One-generation study **[]**; Two-generation study **[]**;
Species/strain:
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration:
Exposure period:
Frequency of treatment:
Post exposure observation period:
Premating exposure period: male: . . . . . , female:
Duration of the test:
Doses:
Control group: Yes **[ ]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Parental: .
NOEL F1 Offspring:
NOEL F2 Offspring:
Results:
General parental toxicity:
Toxicity to offspring:
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance: . . . . . , purity: . . . . . .
Remarks:
Reference:

***5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY**

Species/strain:
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: .
Duration of the test:
Exposure period:
Frequency of treatment:
Doses:
Control group: Yes **[ ]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[]**; Historical **[ ]**
NOEL Maternal Toxicity:
NOEL teratogenicity :
Results:
Maternal general toxicity:
Pregnancy/litter data:

Foetal data:
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance: . . . . . , purity: . . . . . .
Remarks:
Reference:

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

Type:
Results:
Remarks:
Reference:

B. Toxicodynamics, toxicokinetics

Type:
Results:
Remarks:
References:

*** 5.11 EXPERIENCE WITH HUMAN EXPOSURE**

Results: Cyanosis and anemia have been observed in workers involved in the manufacture of Antioxidant 22.
Remarks: Dermal route
Reference: E,I, DuPont de Nemours, 1987

Results: Historically, three incidents involving accidental human overexposure involving Antioxidant 22 have been documented. Skin reactions noted were irritation and a pigmented crust that scaled away in a few days, leaving an erythematous base. Systemic reactions, indicative of skin absorbtion, included profuse perspiration, slow pulse, and a general feeling of anxiety.
Remarks: Data from 1945 does not reflect current industrial practice utilizing Impervious gloves and other personal protective equipment
Reference: Kendrick, M.C., The Medical Bulletin, 1945

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3081-14-9
p-Phenylenediamine, N-1,4-Dimethylpentyl-N'-Phenyl-

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	-36 °C
Decomposition:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Sublimation:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Method:	Not Specified
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	
Reference:	NTP Chemical Repository 1990

*2.2 BOILING POINT

Value:	183 °C
Pressure:	1mm Hg
Decomposition:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Method:	Capillary Melt-Temp Instrument
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	
Reference:	Monsanto Physical Constants of CP25447 (SMP 1977)

†2.3 DENSITY (relative density)

Type:	Bulk density **[ ]**; Density **[X]**; Relative Density **[ ]**
Value:	0.9
Temperature:	27 °C
Method:	Flexsys Standard Method of Analysis FF97.4-1
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	Hydrometer method. Hydrometer must meet standards set in ASTM-E-100
Reference:	ASTM D891-94 method equivalent

*2.4 VAPOUR PRESSURE

Value:	<1.1 x 10(-6) Torr
Temperature:	25°C
Method:	calculated **[ ]**; measured **[X]** Gas Saturation Method, W.F. Spencer and M.M. Cliath, Environ. Sci. Tech. 3, 670 (1969)
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Remarks:	Nitrogen carrier gas, Tenax-GC sorbent, GC analysis
Reference:	Monsanto SRI 8669, SRI International, 1980

*2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

Log Pow:	5.34 log P
Temperature:	22°C
Method:	calculated **[ ]**; measured **[X]** EPA Federal Register Vol. 44, No. 53 (1979)
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Remarks:	Octanol used as solvent
Reference:	Monsanto SRI 8669, SRI International, 1980

***2.6 WATER SOLUBILITY**

A. Solubility

Value:	21 ppm @ pH 5, 0.8 ppm @ pH 9
Temperature:	22°C
Description:	Miscible **[ ]**; Of very high solubility **[ ]**;
	Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble **[ ]**;
	Of low solubility **[ ]**; Of very low solubility[**X]**; Not soluble **[ ]**
Method:	May, W.E., Wasik, S.P., Freeman, D.H., Anal. Chem. 50 (1) 175-178, 1978
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Remarks:	May Method chosen for low-solubility chemicals
Reference:	Monsanto SRI 8669, SRI International, 1980

B. pH Value, pKa Value

pH Value:	Not Applicable

2.7 FLASH POINT *(liquids)*

Value:	182 °C
Type of test:	Closed cup **[ ]**; Open cup **[X]**; Other **[ ]**
Method:	ASTM D 92 Cleveland Open Cup
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Remarks:	No method deviations
Reference:	American Society for Testing and Materials, 1997

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

Value:	
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[ ]**
Remarks:	
Reference:	

3. ENVIRONMENTAL FATE AND PATHWAYS

3.1 STABILITY

***3.1.1 PHOTODEGRADATION**

Type:	Air **[ ]**; Water **[X]**; Soil **[ ]**; Other **[ ]**
Light source:	Sunlight **[X]**; Xenon lamp **[ ]**; Other **[ ]**
Light spectrum:	Natural sunlight, March 7, 1980
Relative intensity:	
Spectrum of substance:	*262* nm
Concentration of Substance:	5ppm
Temperature:	23 °C
Direct photolysis:	
Half life:	2 hours (light) and 4 hours (dark)
Degradation:	
Quantum yield:	
Method:	calculated **[ ]**; measured **[X]**

Direct Photolysis
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance: Santoflex 77 dark liquid, purity: >94%
Remarks:
Reference: Monsanto SR-85-017 SRI International, 1985

Type: Air **[X]**; Water **[ ]**; Soil **[ ]**; Other **[ ]**
Light source: Sunlight **[ ]**; Xenon lamp **[ ]**; Other **[ ]**
Light spectrum: . . . . . nm
Relative intensity: . . . . . *(based on intensity of sunlight)*
Spectrum of substance: . . . . . nm
Concentration of Substance: . . . . . .
Temperature: . . . . . °C
Direct photolysis:
Half life: . . . . . .
Degradation: . . . . . % (weight/weight) after . . . . . (exposure time)
Quantum yield: . . . . . .
Indirect Photolysis:
Type of sensitizer: . . . . . .OH . . .
Concentration of sensitizer: . . 1560000 . . molecule/. cm^3 . . . . .
Rate constant (radical): . . . 125.6992 E-12. . . cm^3/molecule*sec
Degradation: . . . 50% at 1.021 Hrs. . . . . .
Method: calculated **[X]**; AOP Program (v1.89)
measured **[ ]**
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: . molecular structure. . . . . , purity:. . . . . .
Remarks:
Reliability: (2) valid with restrictions
Accepted calculation method
Reference: Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

***3.1.2 STABILITY IN WATER**

Type: Abiotic (hydrolysis) **[X]**; biotic (sediment)**[ ]**
Half life: Not measured
Degradation: 97% at pH 7.0 at 25 °C after 24 hours exposure time
Method: Phase I Hydrolysis Study / ID of Hydrolysis Products
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex 77 dark reddish liquid, purity: >94%
Remarks: Rapid hydrolysis to 4-Hydroxylamine and Benzoquinoneimine-N-phenyl. No test substance detected after 7 days.
Reference: Monsanto ABC-32303 Analytical BioChemistry Labs 1986

***3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)**

Media: Air-biota **[ ]**; Air-biota-sediment-soil-water **[ ]**; Soil-biota **[ ]**; Water-air **[ ]**; Water-biota **[ ]**; Water-soil **[ ]**; Other **[ ]**
Method: Fugacity level I **[ ]**; Fugacity level II **[ ]**; Fugacity level III **[X]**; Fugacity level IV **[ ]**; Other (calculation) **[ ]**; Other (measurement)**[ ]**

Results:

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	0.0609	2.04	1000	2.1e-012
Water	5.53	900	1000	1.65e-013
Soil	31.7	900	1000	1.25e-015
Sediment	62.7	3.6e+003	0	1.11e-013

	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	901	26.6	30	0.885
Water	186	241	6.19	8.04
Soil	1.06e+003	0	35.5	0
Sediment	527	54.7	17.6	1.82

Persistence Time: 1.45e+003 hr
Reaction Time: 1.63e+003 hr
Advection Time: 1.35e+004 hr
Percent Reacted: 89.3
Percent Advected: 10.7

Remarks:
Reliability: (2) valid with restrictions
Accepted calculation method
Reference: Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

***3.5 BIODEGRADATION**

Type: aerobic **[X]**; anaerobic **[ ]**
Inoculum: adapted **[X]**; non-adapted **[ ]**; Sewage/soil/sludge mixture
Concentration of the chemical: 25 mg/l related to COD **[ ]**; DOC **[ ]**; test substance **[X]**
Medium: water **[];** water-sediment **[];** soil **[];** sewage treatment **[X]**
Degradation: 50% of theory after 35 days
Results: readily biodeg. **[];** inherently biodeg. **[X]**; under test condition no biodegradation observed **[ ]**, other **[ ]**
Kinetic . . . . . % in . . . . . (time)
Method: ASTM Proposed Standard for the Determination of the Ultimate Biodegradability of Organic Chemicals, 1979
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance: Santoflex 77 Lot# KL01-04, purity:>94%
Remarks: Sterile controls used – no significant biodegradation noted under sterile conditions. Test run in triplicate.
Reference: Monsanto ES-79-SS-25 MIC Environmental Sciences, 1979

4. ECOTOXICITY

***4.1 ACUTE/PROLONGED TOXICITY TO FISH**

Type of test: static **[X]**; semi-static **[]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]** open-system **[ ]**; closed-system **[X]**
Species: Salmo gairdneri (Rainbow Trout)
Exposure period: 96 hours
Results: LC_{50} (24h) = 51 mg/l

LC_{50} (48h) = 39 mg/l
LC_{50} (72h) = Not Measured
LC_{50} (96h) = 32 mg/l
NOEC = 20 mg/l
LOEC = 32 mg/l

Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 1**
Test substance:. Santoflex 77 dark red liquid Lot #KD05-57 purity:>94%
Remarks: Acetone used as solvent. Water temperature, dissolved oxygen content, and pH monitored throughout study. Data reported at 95% confidence level.
Reference: Monsanto BN-76-254 EG&G Bionomics, 1976

Type of test: static **[X]**; semi-static **[]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**
open-system **[ ]**; closed-system **[X]**
Species: Lepomis machrochirus (Bluegill Sunfish)
Exposure period: 96 hours
Results: LC_{50} (24h) = 261 mg/l
LC_{50} (48h) = 201 mg/l
LC_{50} (72h) = Not Measured
LC_{50} (96h) = 182 mg/l
NOEC = 140 mg/l
LOEC = 180 mg/l

Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 1**
Test substance:. Santoflex 77 dark red liquid Lot #KD05-57 purity:>94%
Remarks: Acetone used as solvent. Water temperature, dissolved oxygen content, and pH monitored throughout study. Data reported at 95% confidence level.
Reference: Monsanto BN-76-254 EG&G Bionomics, 1976

Type of test: static **[X]**; semi-static **[]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**
open-system **[ ]**; closed-system **[X]**
Species: Pimephales promelas (Fathead Minnows)
Exposure period: 96 hours
Results: LC_{50} (24h) = 0.32 mg/l
LC_{50} (48h) = 0.28 mg/l
LC_{50} (72h) = Not Measured
LC_{50} (96h) = 0.28 mg/l
NOEC = Not Determined
LOEC = 0.10 mg/l

Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance:. Santoflex 77 dark red liquid purity 99+%

Remarks: Acetone used as solvent. Water temperature, dissolved oxygen content, and pH monitored throughout study. Data reported at 95% confidence level. Quality check via Antimycin A challenge. Preliminary 72-hour range-finding study used to determine final concentrations.

Reference: Monsanto AB-79-1384361-1a, Analytical BioChemistry Labs, 1979

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**; open-system **[ ]**; closed-system **[X]**

Species: Daphnia magna

Exposure period: 48 hours

Results: EC_{50} (24h) = 0.44 mg/l
EC_{50} (48h) = 0.37 mg/l
NOEC = 10 mg/l

Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance: Santoflex 77 reddish-brown liquid, purity: 99+%

Remarks: Nanograde Acetone used to prepare stock solutions. Water quality parameters (temperature, dissolved oxygen, pH) monitored throughout study. Initial range-finding experiment used to select concentrations. Data reported at 95% confidence level.

Reference: Monsanto AB-79-1384361-1b Analytic Bio-Chemistry Labs, 1979

B. Other aquatic organisms

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**; open-system **[ ]**; closed-system **[X]**

Species: Paratanytarsus parthenogenetica (Midge)

Exposure period: 48 hours

Results: EC_{50} (24h) = 4.4 mg/l
EC_{50} (48h) = 1.7 mg/l
NOEC = 0.56 mg/l

Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance:. Santoflex 77 dark liquid, purity:>94%

Remarks: Stock solutions prepared in acetone. Range-finding experiment run to determine final experimental concentrations. Water quality parameters monitored throughout testing.

Reference: Monsanto AB-81-9AB981014, Analytical BioChemistry Labs, 1981

*4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae

Species: Selenastrum capricornutum (Freshwater alga)

Endpoint: Biomass **[X]**; Growth rate **[ ]**; Other **[ ]**

Exposure period: 96 hours

Results: EC_{50} (24h) = >200 mg/l
EC_{50} (48h) = >120<200 mg/l

EC_{50} (72h) = 86 mg/l
EC_{50} (96h) = 52 mg/l
NOEC = Not Determined
LOEC = Not Determined

Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Selenastrum capricornutum Algal Assay Test 1978
open-system **[ ]**; closed-system **[X]**
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex 77 blackish-red liquid, Lot# KL01-04, purity: 99+%
Remarks: Stock solutions prepared in DMSO. Both cell numbers and decrease of in vivo chlorophyll a measured. Triplicate cultures employed for all test concentrations and for controls. pH monitored throughout test.
Reference: Monsanto BN-79-1384361-2, EG&G Bionomics, 1979

4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

4.5.1 CHRONIC TOXICITY TO FISH (*effects on reproduction, embryo/larva, etc.*)

Type of test: static **[ ]**; semi-static **[ ]**; flow-through **[X]**; other *(e.g. field test)* **[]**; open-system **[ ]**; closed-system **[x]**
Species: Pimephales promelas (Fathead Minnow)
Endpoint: Length of fish **[ ]**; Weight of fish **[X]**;
Reproduction rate **[ ]**; Other **[ ]**
Exposure period: 14 days
Results: EC_{50} (14d) = 0.067 mg/l
NOEC = 0.018 mg/l
LOEC = 0.046 mg/l
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975) and Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex 77 dark liquid, purity: 99+%
Remarks: Stock solutions prepared in Methanol. Water quality parameters monitored throughout test and remained withing acceptable limits. Behavior observations throughout the test indicated that mortality was preceded by surfacing and loss of equilibrium. Weight measurements of surviving fish at the end of the study yielded the following weight percentages of the control group mean weight: 0.018 mg/l = 84%, and 0.046 mg/l = 81%. An apparent lethal threshold of the test substance to fathead minnows was determined to be 0.067 mg/l and was reached after 12 days as indicated by a cessation in mortality from days 12-14.
Reference: Monsanto AB-80-1803058-B1, Analytical BioChemistry Labs, 1981

Type of test: static **[ ]**; semi-static **[ ]**; flow-through **[X]**; other *(e.g. field test)* **[]**; open-system **[ ]**; closed-system **[x]**
Species: Pimephales promelas (Fathead Minnow)
Endpoint: Length of fish **[X]**; Weight of fish **[X]**;
Reproduction rate **[ ]**; Other **[ ]**
Exposure period: 14 days (336 hours)
Results: LC_{50} (24h) = 0.07 mg/l

LC_{50} (96h) = 0.06 mg/l
LC_{50} (14d) = 0.05 mg/l
NOEC = Not Determined
LOEC = Not Determined

Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975) and Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance: Santoflex 77 dark reddish liquid, purity: 99+%

Remarks: Stock solutions prepared in acetone and stabilized with ascorbic acid. Water quality parameters monitored throughout test and remained withing acceptable limits. Samples analyzed for concentration of test article varied widely. This variability was attributed to the instability of the test compound in water and to incomplete dispersion. Nominal concentrations of test compound were 0.00, 0.03, 0.06, 0.12, 0.25 and 0.50 mg/l. LC50s were recorded at 24, 96 and 336 hours. At the time the test was terminated, no mortalities had occurred during the preceeding 48 hours.

Reference: Monsanto SR-80-1803058-A1, SRI International, 1981

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Type: LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**

Species/strain: Sprague-Dawley Albino Rats

Value: 730 mg/kg b.w.
Discriminating dose: 794 mg/kg

Method: Defined Lethal Dose

GLP: Yes **[ ]** No **[ ]** ? **[x]** **Klimisch 2**

Test substance: Santoflex 77, Lot # KC01-04 , purity:>94%

Remarks: Groups of male and female rats were fed either 501, 631, 704 or 1000 mg/kg of the undiluted test substance as a single oral dose by gavage. Clinical signs of toxicity included reduced appetite and activity – for to six days in survivors – followed by increasing weakness, collapse and death. Gross autopsy findings on decedents included hemorrhagic areas of the lungs, liver discoloration and acute gastrointestinal inflammation. Survivors were sacrificed after 10 days. All viscera examined appeared normal.

Reference: Monsanto Y-73-168 Younger Laboratories, 1973

5.1.2 ACUTE INHALATION TOXICITY

Type: LC_0 **[X]**; LC_{100} **[ ]**; LC_{50} **[ ]**; LCL_0 **[ ]**; Other **[ ]**

Species/strain: Sprague-Dawley Albino rats

Exposure time: 6 hours w/10 day observation period

Value: Sample did not vaporize

Method: Ambient Temperature Inhalation

GLP: Yes **[ ]** No **[ ]** ? **[x]** **Klimisch 2**

Test substance: Santoflex 77 Lot # KC01-04, purity: >94%

Remarks: Male rats were exposed to the test article in an inhalation chamber for a period of six hours at ambient temperature. The initial sample size of the test article was 133 grams. At the end of six hours, the sample was reweighed and found to be 133 grams, and no sample was recovered from the chamber air condenser. Santoflex 77 did not vaporize under the test conditions. No animal experienced any symptoms of toxicity. The 10 day observation period was uneventful, and all animals survived to sacrifice with no noted ill-effects. Autopsy findings were that all viscera examined appeared normal.

Reference: Monsanto Y-73-168 Younger Laboratories, 1973

5.1.3 ACUTE DERMAL TOXICITY

Type: LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**

Species/strain: New Zealand Albino Rabbits

Value: >3160 mg/kg b.w.

Method: Defined Lethal Dose

GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**

Test substance: Santoflex 77, Lot # KC01-04, purity: >94%

Remarks: The undiluted test substance was applied to the shaved skin of male and female rabbits for a period of 24 hours, followed by a 14 day recovery period. Dosages were 1260, 2000, 3160, 5010 or 7940 mg/kg. Clinical signs of toxicity were reduced appetite and activity – three to seven days in survivors – followed by increasing weakness, collapse and death. Gross autopsy findings on decedents included lung hyperemia, liver discoloration, enlarged gall bladder and gastrointestinal inflammation. Survivors were sacrificed following the recovery period. All viscera appeared normal on all but two animals, which exhibited a slight discoloration of both liver and kidneys.

Reference: Monsanto Y-73-168 Younger Laboratories, 1973

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

Species/strain: New Zealand Albino Rabbits

Results: Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[X]**

Classification: Highly corrosive (causes severe burns) **[ ]**; Corrosive (causes burns) **[ ]**; Irritating **[ ]**; Not irritating **[X]**

Method: Primary Skin Irritation

GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**

Test substance: Santoflex 77 Lot #KC01-04, purity:>94%

Remarks: 0.5 ml of the undiluted test substance was applied to the shaved skin of six male and female rabbits. Irritation was scored on a scale of 0-4 for both erythema and edema. The 24 hour score for all animals was 0.0, indicating the the test substance was non-irritating. Observations noted was a slight defatting effect on the skin, with mild flaking after 7-10 days.

Reference: Monsanto Y-73-168 Younger Laboratories, 1973

5.2.2 EYE IRRITATION/CORROSION

Species/strain: New Zealand Albino Rabbits

Results Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**;

Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[X]**;
Not irritating **[ ]**

Classification: Irritating **[X]**; Not irritating **[ ]**; Risk of serious damage to eyes **[ ]**

Method: Draize

GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**

Test substance: Santoflex 77 Lot # KC01-04, purity: >94%

Remarks: 0.1 ml of the undiluted test substance was applied to the eyes of rabbits. Irritation was assessed at 1, 24, 48, 72 and 168-hour intervals on the basis of irritation to the cornea, iris and conjunctivae. Immediate findings were slight discomfort. 1-hour findings were slight erythema, very slight edema and copious discharge. 24-hour score was 10.0, 48-hour score was 9.3, 72-hour score was 6.3 and 168-hour score was 0.0. The 24/48/72 hour average score was 8.5 for a classification as a "slight" acute eye irritant.

Reference: Monsanto Y-73-168 Younger Laboratories, 1973

5.3 SKIN SENSITISATION

Type:

Species/strain:

Results: Sensitizing **[ ]**; Not sensitizing **[ ]**; Ambiguous **[ ]**

Classification: *(if possible, according to EC Directive 67/548/EEC)*
Sensitizing **[ ]**; Not sensitizing **[ ]**

Method:

GLP: Yes **[ ]** No **[ ]** ? **[ ]**

Test substance: . . . . . , purity: . . . . . .

Remarks:

Reference:

*5.4 REPEATED DOSE TOXICITY

Species/strain: Sprague-Dawley CD Rats

Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**

Route of Administration: Oral/Dietary

Exposure period: 30 days

Frequency of treatment: Daily

Post exposure observation period:

Dose: 0, 100, 300, 500, 1000 and 2000 ppm

Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**

NOEL: 100 ppm for males, 300 ppm for females

LOEL: Not Determined

Results: In a 30-day range-finding study that preceeded a 90-day study, the test substance was administered orally, via dietary admixture, to groups of male and female rats (5/sex/group). Control animals received the standard laboratory diet. Physical observations, body weight and food consumption measurements were performed on all animals pretest and at selected intervals during the study. Hematology and chemistry determinations were performed on all animals at study termination. There were no mortalities during the course of the study. After four weeks of treatment, all animals were sacrificed, selected organs were weighed, and organ/body weight ratios were calculated. Complete postmortem examinations were conducted on all animals. Differences from control in mean body weights were statistically significant at 500 ppm and 1000 ppm males and in 2000

ppm males and females. Differences from control in mean body weight/body weight gain suggested a treatment-related effect in males at dose levels at and above 300 ppm, and in females at and above 1000 ppm. Food consumption values in Week 1 were reduced for males at 500 ppm and above, and for females at 300 ppm and above. Food consumption at Weeks 3-4 was comparable to controls. Males and females at the two highest dose levels exhibited increased mean platelet counts following four weeks of treatment. Males in these groups also exhibited increased mean erythrocyte. The mean hematology values for males and females in all treatment groups were comparable to controls. Alterations in several clinical chemistry parameters were noted for higher dose levels. Mean terminal body weights were reduced at the two highest dose levels in females, and at the three highest dose levels in males. While several organs in treated males and females exhibited alterations in either mean absolute or relative weights, these changes were considered secondary effects and not indicative of significant organ toxicity. Gross pathological examination did not reveal any effects that were considered treatment-related.

Method: Dunnett, C.W., A Multiple Comparison Procedure for Comparing Several Treatments with a Control, Jour. Am. Stat. Assoc. 50: 1096-1121, 1955

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance:. Santoflex 77 Lot# KJ01-03, purity: 99+% active

Reference: Monsanto BD-87-146 Bio/dynamics Labs, 1987

Species/strain: Sprague-Dawley CD Rats

Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**

Route of Administration: Oral/Dietary

Exposure period: 90 days

Frequency of treatment: Daily

Post exposure observation period:

Dose: Males: 0, 100, 250 and 500 ppm Females: 0, 250, 500 and 750 ppm

Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**

NOEL: 100 ppm for males, not established for females

LOEL: Not Determined

Results: The test substance was administered orally, via dietary admixture, to groups of male and female rats (10/sex/group). Control animals received the standard laboratory diet. Physical observations, body weight and food consumption measurements were performed on all animals pretest and at selected intervals during the study. Hematology and chemistry determinations were performed on all animals at Months 1.5 and 3. There were no mortalities during the course of the study. After three months of treatment, all animals were sacrificed, selected organs were weighed, and organ/body and organ/brain weight ratios were calculated. Complete postmortem examinations were conducted on all animals. Histopathological evaluation of selected tissues was performed on all control and high-dose animals. The lungs, spleen, liver and kidneys were examined microscopically for all animals in all groups. Mean body weights and mean body weight gains were reduced in males at 250 and 500 ppm,

and in all treated females. Overall, mean food consumption values for all treated groups were comparable to controls. Several clinical chemistry parameters exhibited statistically significant differences from control. Alkaline phosphatase was elevated in the 500 ppm males and 750 ppm females at Month 3. Mean serum glutamic oxaloacetic transaminase levels were significantly reduced in the 100, 250 and 500 ppm males at Month 1.5 but not at Month 3. Mean serum glutamic pyruvic transaminase was reduced in the 500 and 750 ppm females at Month 3. Several organs in the treated males and females exhibited alterations in mean absolute and/or relative (to body or brain) weight data. However, these alterations were generally consistent with the reductions noted in body weight data and were considered secondary effects which were not considered indicative of significant organ toxicity. There were no treatment-related findings noted in mortality, physical observations, opthalmoscopic, hematology, organ weight or gross and microscopic pathology.

Method: OECD Guidelines for Testing of Chemicals, Section 453, 1981 and USEPA TSCA Section 4(a) Test Rules, 1982

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance:. Santoflex 77 Lot# KJ01-03, purity: 99+% active

Reference: Monsanto BD-87-147 Bio/dynamics Labs, 1989

Species/strain: Charles River Albino rats

Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**

Route of Administration: Oral/Dietary

Exposure period: 2 years

Frequency of treatment: Daily

Post exposure observation period:

Dose: 0, 30, 100 or 300 ppm

Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**

NOEL: 30 ppm

LOEL: 100 ppm

Results: A two-year chronic oral toxicity study was conducted on groups of 400 CD Outbred rats (50/sex/dose) at dietary levels ranging from 0-300 ppm. Reductions in body weights and body weight gains were noted for males and females at the 300 ppm dose throughout the investigation. Body weights of females fed 100 ppm were reduced during the first 7 weeks, and for 100 ppm males for the first 4 weeks. After those intervals, body weights compared favorably with controls. 30 ppm animals had body weights and weight gains that compared favorably with controls. Frequency and distribution of deaths during the investigation for all dose levels was similar to controls. Gross pathological examination of animals that died during the study did not reveal any relation between death and exposure to the test substance. No unusual behavioral reactions were noted in dosed animals during the course of the study. Results of hematologic studies conducted – total and differential leukocyte count, erythrocyte count, hemoglobin concentration, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration – were either similar to, or within the range of expected values for this strain of albino rats of this age and in this laboratory. Results of clinical blood chemistry studies (SGPT, BUN, SGOT, Fasting

Blood Glucose Concentration, SAP) and of urinalyses (glucose, albumin, microscopic elements, pH and specific gravity) conducted showed similar results between control and test animals. Gross pathological examinations of animals sacrificed at 24 months revealed similar findings between test and control animals. Histopathological examinations of tissues and organs from the control and 300 ppm animals sacrificed at 24 months showed no treatment-related lesions. Microscopic examination of suspect neoplasms among all sacrificed animals and all animals that died during the study were conducted. No differences were noted between test and control rats as to the organ system involved, the type or the classification of neoplasms. The spectrum of neoplasms observed compared favorably to historical data at this laboratory for rats of this strain and age. At 17.5 months of testing, tetracycline HCl was added to the diets of all groups (30g/kg of diet) for a two-week period to treat a severe respiratory infection which caused an increase in mortality in both control and treated animals.

Method: 2-Year Chronic Oral Toxicity IBT Protocol # 622-05400B (1974)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex 77 reddish liquid Lot# KD05-57, purity: 99+% active
Reference: Monsanto BTL-74-27, Industrial Bio-Test Labs, 1978

*5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

Type: Ames Reverse Bacterial Mutation
System of testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537
Concentration: 0.01, 0.04, 0.2, 1, 3, 10, 40 and 200 micrograms/plate
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:
Cytotoxicity conc: With metabolic activation: 200 micrograms/plate
Without metabolic activation: 10 micrograms/plate
Precipitation conc: 1 microgram/plate
Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[X]**
Without metabolic activation:	**[ ]**	**[ ]**	**[X]**

Method: Ames, B.N., McCann, J. and Yamaski, E. Methods for Detecting Carcinogens and Mutagens with the Salmonella Mammalian-Microsome Test. Mutat. Res. 31, 347-364, 1975
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex 77 Lot# 7/31/85WGK, purity: 99+% active
Remarks: Santoflex 77 was tested in Ames/Salmonella plate incorporation assays using the tester strains TA98, TA100, TA1535 and TA1537 in the presence and absence of an Aroclor-induced rat liver mammalian metabolic activation system (S-9 Mix). No mutagenic activity was observed for the test compound in any of these assays. Toxicity of the test compound was significantly reduced in the presence of the S-9 Mix.
Reference: Monsanto ML-85-242 Environmental Health , 1985

B. NON-BACTERIAL IN VITRO TEST

Type:	Mammalian Cell Gene Forward Mutation Assay
System of testing:	L5178Y Mouse Lymphoma cells
Concentration:	0.002, 0.004, 0.008, 0.016 (without activation) 0.002, 0.004, 0.008, 0.016, 0.032 (with activation)
Metabolic activation:	With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:	
Cytotoxicity conc:	With metabolic activation: 0.032 ug/ml Without metabolic activation: 0.016 ug/ml
Precipitation conc:	Not determined
Genotoxic effects:	+ ? - With metabolic activation: **[ ] [ ] [X]** Without metabolic activation: **[ ] [ ] [X]**
Method:	Clive and Spector, Mutation Research 31:17-29 (1975)
GLP:	Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance:	CP-25477 (Santoflex 77) dark liquid, purity >94%
Remarks:	The test article was evaluated for specific locus forward mutation in the L5178Y Thymidine Kinase (TK) mouse lymphoma cell assay. Stock solutions were prepared in DMSO. DMSO was used as the negative control. EMS was used as the positive control without activation and DMN was used as the positive control with activation. The test article was found to be negative
Reference:	Monsanto BIO-76-246 Litton Bionetics, 1976

Type:	*In vitro* Unscheduled DNA Synthesis (UDS)
System of testing:	Primary rat hepatocyte cultures (Fischer-344 strain)
Concentration:	0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, 50, 100, 500, 1000 ug/ml
Metabolic activation:	With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:	
Cytotoxicity conc:	Preliminary Assay: 50 ug/ml Replicate Assay: 5 ug/ml
Precipitation conc:	Separation (two layers) at 1000 ug/ml
Genotoxic effects:	+ ? - **[ ] [ ] [X]**
Method:	Williams, G.M., Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures, Cancer Research 37, pp. 1845-1851 (1977)
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:	Santoflex 77 liquid produced 07/31/85, purity 99+% active
Remarks:	Acetone (1%) used as solvent and diluent. Primary rat liver cell cultures derived from the livers of two adult male rats. The positive control was 2-AAF, the solvent control was acetone in the preliminary assay and DMSO in the replicate assay. The percentage of cells in repair was calculated as the percentage of cells with at least 5 net grains/nucleus. 150 cells were scored for each concentration reported for each experiment. The net grain counts were negative at each concentration of the test compound, in the solvent control and in the medium control, in contrast to the strong positive response produced by the positive control 2-AAF in both experiments. These results indicate that Santoflex 77 is not a genotoxic agent under the conditions of the in vitro rat hepatocyte DNA repair assay.
Reference:	Monsanto SR-85-250, SRI International, 1986

*** 5.6 GENETIC TOXICITY IN VIVO**

Type:
Species/strain:
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration:
Exposure period:
Doses:
Results:
Effect on mitotic
index or P/N ratio:
Genotoxic effects: + ? -
[ ] [ ] [ ]
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance:
Remarks:
Reference:

5.7 CARCINOGENICITY

Species/strain:
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration:
Exposure period:
Frequency of treatment:
Postexposure observation period:
Doses:
Control group: Yes **[ ]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results:
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance:` . . . . . , purity: . . . . . .
Remarks:
Reference:

***5.8 TOXICITY TO REPRODUCTION**

Type: Fertility **[X]**; One-generation study **[ ]**; Two-generation study **[ ]**;
Other **[X]** Three Generation Study
Species/strain: Charles River Albino Rats
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral/Dietary
Exposure period: Premating, throughout mating, gestation and lactation
Frequency of treatment: Daily
Post exposure observation period: Not Determined
Premating exposure period: male: F0 – 14 wks F1- 14 wks F2 – 18 wks
female: F0 – 14 wks F1 – 14 wks F2 – 18 wks
Duration of the test: F0 – 23 wks F1 – 23 wks F2 – 26 wks
Doses: 0, 30, 100 or 300 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Parental: 30 ppm (based on reduced body weight gain)

NOEL F1 Offspring: 30 ppm (based on reduced pup survival)
NOEL F2 Offspring: 30 ppm (based on reduced pup survival)
Results: Santoflex 77 was administered to three successive generations of rats at dose levels of 0, 30, 100 or 300 ppm. Dose levels were selected on the basis of results from a previous 2-year chronic oral feeding study. No adverse effects on mating or fertility indices were noted in any of the treated animals. Reduced survival of offspring was observed in the mid- to high-dose groups. Evidence of parental toxicity was also present as indicated by reduced body weights of mid-to high-dose animals
General parental toxicity: Reduced body weights and mean body weight gains were noted for the 100 and 300 ppm males and females. No other treatment-related effects were evident in results of clinical blood chemistry studies and urinalyses between the control groups and the treated animals.
Toxicity to offspring: A small but statistically significant reduction in the survival rates of pups was noted in the 100 ppm and 300 ppm groups.
Method: 3-Generation Reproductive Toxicity IBT Protocol # 622-05400C (1974)
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance: Santoflex 77 dark red liquid Lot# KD05-57, purity: 99+% active
Remarks: Protocol similar to Monsanto BTL-74-27, Industrial Bio-Test Labs, 1978
Reference: Monsanto BTL-76-145, Industrial Bio-Test Labs, 1976

***5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY**

Species/strain: Charles River CD Albino Rats
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Oral gavage
Duration of the test: 25 days from mating to last C-section
Exposure period: Day 6-15 of gestation
Frequency of treatment: Daily, as a single oral dose at a volume of 5 ml/kg
Doses: 25, 75 and 150 mg/kg/day
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL Maternal Toxicity: 25 mg/kg/day
NOEL teratogenicity : 150 mg/kg/day
Results: Groups of 25 mated CD rats were assigned to one control group and three treatment groups to determine the teratogenic potential of the test substance. Dosage levels of 25, 75 and 150 mg/kg/day were administered orally by gavage as a single daily dose on Days 6-15 of gestation. The control group received the corn oil vehicle only. Cesarean sections were performed on all surviving females on gestation Day 20, and the fetuses removed for teratologic evaluation.
Maternal general toxicity: Toxicity in the dams was apparent at the 75 and 150 mg/kg/day dosage levels. Parameters adversely affected were maternal survival, appearance, behavior and body weight gain. Four of the 150 mg/kg/day females and one 75 mg/kg/day female died between gestation Days 16-17. Control animals and the low dose group had 100% survival. Antemortem abnormalities in the decedents included dried blood around and/or expelled from the vaginal orifice, blood under the cage, stained, wet or matted coat, hypothermia and ptyalism. There were no treatment-related gross internal lesions evident. No effect on Cesarean section observations was noted in the dams at any dosage level.
Pregnancy/litter data: No obvious differences were noted between the

Treated groups and the control group.
Foetal data: Malformations that were observed in the treated groups occurred in low incidence and were not considered treatment-related. One high-dose fetus had anophthalmia, one mid-dose and two control group fetuses had microphthalmia, and another mid-dose fetus had ectopia cordia and sternoschisis. There were no adverse effects on the fetal parameters examined (survival, growth, morphological development) at dose levels at or below 150 mg/kg/day.

Method: OECD Guidelines for Testing of Chemicals No. 414 "Teratogenicity" 1981, and TSCA Health Effects Guidelines "Teratogenicity Study" 1982

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance: Santoflex 77 red-brown liquid Lot# 25477, purity: 99+% active

Remarks: Based on the results, the test article did not induce developmental toxicity In the offspring of Charles Rived CD rats under the test conditions.

Reference: Monsanto IR-85-290 International Research and Development, 1986

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

Type: Immunotoxicity – Repeated Insult Patch Testing
Modified Schwartz Method and Shelanski Method

Results: Several studies were run using human volunteers to determine the potential for Santoflex 77 to cause allergic skin reactions in compounded rubber stocks. Loading of the test article was from 0.5 to 3 phr (parts per hundred rubber) in a typical B-1 Masterbatch. Some study results indicated that the test article caused no primary irritation and no allergic response, while other study results were positive for sensitization.

Remarks: Differences in responses may be due to the presence of other chemicals in the B-1 masterbatch formulations.

Reference: Monsanto SH-61-17, Industrial Biology Labs, 1961
Monsanto SH-63-10, Industrial Biology Labs, 1963
Monsanto SH-64-4, Industrial Biology Labs, 1964
Monsanto SH-64-5, Industrial Biology Labs, 1964
Monsanto SH-73-12, Industrial Biology Labs, 1973

B. Toxicodynamics, toxicokinetics

Type: *(e.g. toxicodynamics, toxicokinetics)*
Results:
Remarks:
References:

*** 5.11 EXPERIENCE WITH HUMAN EXPOSURE**

Results:
Remarks:
Reference:

6. REFERENCES

1. United States National Toxicology Program, November 6, 1990
2. Monsanto Physical Constants of CP25447. Standard Manufacturing Process Manual, July 1977
3. American Society for Testing and Materials, 1997
4. Monsanto SRI 8669, Selected Environmental Fate Studies of Nine Chemical Compounds, SRI International, August 20, 1980

5. USEPA federal Register Volume 44, No. 53, March 16, 1979, pp. 16 and 255

6. American Society for Testing and Materials, D 92, Standard Test Method for Flash and Fire Points by Cleveland Open Cup, 1997

7. Monsanto Report ABC-32303, Santoflex 77 Phase I Hydrolysis Study: Identification of Hydrolysis Products, Analytical BioChemistry Laboratories, January 15, 1986

8. Monsanto ES-79-SS-25, Environmental Persistence Screening of Selected Rubber Chemicals, Monsanto Industrial Chemicals Environmental Sciences, December 28, 1979

9. American Society for Testing and Materials, Draft Method No. 2, ASTM Committee E35.24, August 1979

10. Monsanto BN-76-254 Acute (96 Hour) Toxicity of Santoflex 77 to Rainbow Trout, EG&G Bionomics Aquatic Toxicity Laboratory, December 1976

11. Monsanto BN-76-254 Acute (96 Hour) Toxicity of Santoflex 77 to Bluegill Sunfish, EG&G Bionomics Aquatic Toxicity laboratory, December 1976

12. Monsanto AB-79-1384361-1b Acute Toxicity of Santoflex 77 to Daphnia magna, Analytical BioChemistry Laboratories, August 27, 1979

13. Monsanto AB-79-1384361-1a Acute Toxicity of Santoflex 77 to Fathead Minnows, Analytical BioChemistry Laboratories, August 27, 1979

14. Monsanto BN-79-1384361-2 Toxicity of Santoflex 77 to the freshwater alga Selenastrum capricornutum, EG&G Bionomics Marine Research Laboratory, August 1979

15. Monsanto AB-81-9AB981014, Acute Toxicity of Santoflex 77 to Midge, Analytical BioChemistry Laboratories, August 19, 1981

16. Gettings, A.V and W.J. Adams. 1980. Method for Conducting Acute Toxicity Tests with the Midge Paratanytarsus parthenogenetica. Monsanto Industrial Chemicals Company, Report ES-81-M-1

17. C.E. Stephan, Chairman, Committee on Methods for Toxicity Tests with Aquatic Organisms, US EPA, 1975

18. Monsanto AB-80-1803058-B1, Flow-Through Bioassay Final Report: Dynamic Acute Toxicity of Santoflex 77 to Fathead Minnows, Analytical BioChemsitry Laboratories, January 20, 1981

19. Monsanto SR-80-1803085-A1, Time Independent Toxicity Study on Santoflex 77 using Fathead Minnows as the Test Organism, SRI International, September 8, 1981

20. Monsanto Y-73-168, Toxicological Examination of CP-25477 (Santoflex 77) for Acute Oral and Dermal Toxicity, Younger Laboratories, October 9, 1973

21. Monsanto Y-73-168, Toxicological Examination of CP-25477 (Santoflex 77) for Ambient Temperature Inhalation Toxicity, Younger Laboratories, October 9, 1973

22. Monsanto Y-73-168, Toxicological Examination of CP-25477 (Santoflex 77) for Acute Eye and Primary Skin Irritation, Younger Laboratories, October 9, 1973

23. Monsanto BD-87-146, A 4 Week Range-Finding Toxicity Study with Santoflex 77 in the Rat Via Dietary Admixture, Bio/dynamics, Inc. June 14, 1989

24. Monsanto BD-87-147, A Subchronic 3-Month Oral Toxicity Study with Santoflex 77 in the Rat Via Dietary Admixture, Bio/dynamics, Inc. April 28, 1989

25. Monsanto BTL-74-27, Two-Year Chronic Oral Toxicity Study with Santoflex 77 in Albino Rats, Industrial Bio-Test Laboratories, Inc. November 27, 1978

26. Monsanto ML-85-242, Ames/Salmonella Mutagenicity Assay of Santoflex 77, Monsanto Environmental Health Laboratory, February 18, 1986

27. Monsanto SR-85-250, Evaluation of the Potential of Santoflex 77 to Induce Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures, SRI International, May 23, 1986

28. OECD Guidelines for Testing of Chemicals: No. 414, Teratogenicity, adopted May 1981

29. US EPA Report 560/6-82-001, TSCA Health Effects Test Guidelines for Teratogenicity Studies, August 1982

30. Monsanto IR-85-290, Teratology Study in Rats with Santoflex 77, International Research and Development Corporation, April 1, 1986

31. Monsanto SH-61-17, Repeated Insult Patch Tests of Antidegradants, Industrial Biology Laboratories, Inc. May, 1961

32. Monsanto SH-63-10, Modified Schwartz Patch Test Study of Monsanto Rubber Samples, Industrial Biology Laboratories, Inc., November 8, 1963

33. Monsanto SH-64-4, Repeat Insult Patch Test on Vulcanized Rubbers, Industrial Biology Laboratories, May 5, 1964

34. Monsanto SH-64-5, Dermatitic Studies of Hexyl- and Heptyl-PPDs in Rubber, Industrial Biology Laboratories, March 1964

35. Monsanto SH-73-12, Repeat Insult Patch Test with Uncured Rubbers, Industrial Biology Laboratories, April 1973

3081-01-4
p-Phenylenediamine, N-(1,4-dimethylpentyl)-N'-phenyl-

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	32.4°C for highly purified (99+%) Otherwise, room temperature viscous liquid
Decomposition:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Sublimation:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Method:	Crystallizing Point
GLP:	Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Remarks:	Physical Constants, Flexsys SMP, R.L. Wright (1982)
Reference:	Flexsys 7PPD Standard Manufacturing Process

*2.2 BOILING POINT

Value:	231 °C
Pressure:	at 3.5 mm Hg
Decomposition:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Method:	Instrumental – Differential Scanning Calorimeter (DSC)
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Remarks:	Physical Constants, Flexsys SMP, R.L. Wright (1982)
Reference:	L.M. Baclawski Notebook #2355311 (1982)

†2.3 DENSITY (relative density)

Type:	Bulk density **[ ]**; Density **[X]**; Relative Density **[ ]**
Value:	1.0
Temperature:	20 °C
Method:	Flexsys Standard Method of Analysis FF97.4-1
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Remarks:	Hydrometer method. Hydrometer must meet standards set in ASTM-E-100
Reference:	Flexsys 7PPD Standard Manufacturing Specifications

*2.4 VAPOUR PRESSURE

Value:	1.25 x 10(-10) mm Hg
Temperature:	25 °C
Method:	calculated **[X]**; measured **[ ]** Antoine Equasion.
GLP:	Yes **[ ]** No **[X]** ? **[ ]** **Klimisch 2**
Remarks:	None
Reference:	Monsanto Toxicology Profile, Santoflex 14, C.E. Healy 1993

*2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

Log Pow:	5.17
Temperature:	Not Applicable
Method:	calculated **[X]**; measured **[ ]** **Klimisch 2** . SRC LogKow (KowWin) Program 1995
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	None

Reference: Meylan, W.M. and. P.H. Howard, 1995 J. Pharm. Sci. 84: 83-92

*2.6 WATER SOLUBILITY

A. Solubility

Value: 0.67 mg/l in pH 7.0 deionized water
Temperature: 25°C
Description: Miscible **[ ]**; Of very high solubility **[ ]**;
Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble **[ ]**;
Of low solubility **[ ]**; Of very low solubility **[X]**; Not soluble **[]**
Method: Saturated Solution/GC Analysis
GLP: Yes **[X]** No **[]** ? **[ ]** **Klimisch 1**
Remarks: Preliminary solubility study for Phase I Hydrolysis
Reference: Monsanto ABC 32305, Analytical Bio-Chemistry Labs, 1986

B. pH Value, pKa Value

pH Value: Not Applicable
Concentration:
Temperature:
Method: .
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
pKa value
Remarks:
Reference:

2.11 OXIDISING PROPERTIES

Results: Maximum burning rate equal or higher than reference mixture**[ ]**;
Vigorous reaction in preliminary test **[ ]**;
No oxidising properties **[X]**; Other **[ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Remarks:
Reference:

†2.12 OXIDATION: REDUCTION POTENTIAL

Value: Not Applicable
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Remarks:
Reference:

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

Value:
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Remarks:
Reference:

B. Other data

Results: .
Remarks:
Reference:

3. ENVIRONMENTAL FATE AND PATHWAYS

*3.1.1 PHOTODEGRADATION

Type: Air **[X]**; Water **[ ]**; Soil **[ ]**; Other **[ ]**
Light source: Sunlight **[ ]**; Xenon lamp **[ ]**; Other **[ ]**
Light spectrum: . . . . . nm
Relative intensity: . . . . . *(based on intensity of sunlight)*
Spectrum of substance: . . . . . nm
Concentration of Substance: . . . . . .
Temperature: . . . . . °C
Direct photolysis:
Half life: . . . . . .
Degradation: . . . . . % (weight/weight) after . . . . . (exposure time)
Quantum yield: . . . . . .
Indirect Photolysis:
Type of sensitizer: . . . . . OH . .
Concentration of sensitizer: . .1560000 molecule/. cm^3 . . . . . .
Rate constant (radical): . . . 227.9058 E-12. cm^3/molecule*sec
Degradation: . . . . . 50% at 0.563 Hrs . . .
Method: calculated **[X]**; AOP Program (v1.89)
measured **[ ]**

GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance:. molecular structure, purity:. . . . . .
Remarks:
Reliability: (2) valid with restrictions
Accepted calculation method
Reference: Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

*3.1.2 STABILITY IN WATER

Type: Abiotic (hydrolysis) **[X]**; biotic (sediment)**[ ]**
Half life: Not Measured
Degradation: 96% at pH 7.0 at 25 °C after 24 Hours
Method: Extraction, ABC Protocol M-8305 (1986)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex 14 purple liquid, Lot# KD09-813, purity:>95%
Remarks: No test substance detected at seven days. Hydrolysis products identified by GC analysis as 4-hydroxydiphenylamine (35%) and Benzoquinoneimine-n-phenyl (65%). Stock solution in acetone.
Reference: Monsanto ABC 32305, Analytical Bio-Chemistry Labs, 1986

***3.2 MONITORING DATA (ENVIRONMENTAL)** .....

Type of Measurement: Background **[ ]**; At contaminated site **[ ]**; Other **[ ]**
Media: .....
Results: .....
Remarks: .....
Reference: .....

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION

*3.3.1 TRANSPORT

Type: Adsorption **[ ]**; Desorption **[ ]**; Volatility **[ ]**; Other **[ ]**
Media:
Method:
Results:
Remarks:
Reference:

*3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota **[ ]**; Air-biota-sediment-soil-water **[ ]**; Soil-biota **[ ]**; Water-air **[ ]**; Water-biota **[ ]**; Water-soil **[ ]**; Other **[ ]**
Method: Fugacity level I **[ ]**; Fugacity level II **[ ]**; Fugacity level III **[X]**; Fugacity level IV **[ ]**; Other (calculation) **[ ]**
Results:

C

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	0.027	1.13	1000	7.19e-013
Water	15.2	900	1000	3.5e-014
Soil	57.5	900	1000	1.11e-015
Sediment	27.2	3.6e+003	0	2.36e-014

	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	531	8.64	17.7	0.288
Water	375	487	12.5	16.2
Soil	1.41e+003	0	47.1	0
Sediment	168	17.4	5.58	0.58

Persistence Time: 1.06e+003 hr
Reaction Time: 1.28e+003 hr
Advection Time: 6.23e+003 hr
Percent Reacted: 82.9
Percent Advected: 17.1

Remarks:
Reliability: (2) valid with restrictions
Accepted calculation method
Reference: Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

***3.5 BIODEGRADATION**

Type:	aerobic **[X]**; anaerobic **[ ]**
Inoculum:	adapted **[X]**; non-adapted **[ ]**;
Concentration of the chemical: 20.0 mg/l related to COD **[X]**; DOC**[ ]**; test substance**[ ]**	
Medium:	water **[ ];** water-sediment **[ ];** soil **[ ];** sewage treatment **[X]**
Degradation:	0 % after 35 days
Results:	readily biodeg. **[ ];** inherently biodeg. **[]**; under test condition no biodegradation observed **[X]**, other **[ ]**
Kinetic	
Method:	ASTM Draft 3 Proposed Standard Practice for the Determination Of the Ultimate Biodegradation of Organic Chemicals (1980).
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:	Santoflex 14 purple liquid Lot#KA01-07, purity: >95%
Remarks:	Shake Flask carbon dioxide evolution test. Glucose and Sodium Citrate used as positive controls.
Reference:	Monsanto ES-80-SS-48 MIC Environmental Sciences 1981

4. ECOTOXICITY

***4.1 ACUTE/PROLONGED TOXICITY TO FISH**

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]** open-system **[ ]**; closed-system **[X]**
Species:	Salmo gairdneri (Rainbow Trout)
Exposure period:	96 Hours
Results:	LC_{50} (24h) = >1.00 mg/l LC_{50} (48h) = 0.70 mg/l LC_{50} (72h) = Not Determined LC_{50} (96h) = 0.42 mg/l NOEC = 0.18 mg/l LOEC = Not Determined
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:	Santoflex 14 purple liquid, purity:>95%
Remarks:	Stock solutions prepared in nanograde Acetone. Water quality parameters (pH, temperature, dissolved oxygen content) monitored throughout test. Test fish challenged with Antimycin A. Data reported at 95% confidence level.
Reference:	Monsanto ABC 30687, Analytical Bio-Chemistry Labs, 1983

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]** open-system **[ ]**; closed-system **[X]**
Species:	Lepomis macrochirus (Bluegill Sunfish)
Exposure period:	96 Hours
Results:	LC_{50} (24h) = 0.38 mg/l LC_{50} (48h) = 0.30 mg/l LC_{50} (72h) = Not Determined LC_{50} (96h) = 0.30 mg/l NOEC = 0.18 mg/l LOEC = Not Determined
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:	Santoflex 14 purple liquid , purity:>95%
Remarks:	Stock solutions prepared in nanograde Acetone. Water quality parameters (pH, temperature, dissolved oxygen content) monitored throughout test. Test fish challenged with Antimycin A. Data reported at 95% confidence level.
Reference:	Monsanto ABC 30686, Analytical Bio-Chemistry Labs, 1983

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]** open-system **[ ]**; closed-system **[X]**
Species:	Pimephales promelas (Fathead Minnows)
Exposure period:	96 Hours
Results:	LC_{50} (24h) = 1.30 mg/l LC_{50} (48h) = 1.30 mg/l LC_{50} (72h) = Not Determined LC_{50} (96h) = 1.10 mg/l NOEC = 0.32 mg/l LOEC = Not Determined
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:	Santoflex 14 purple liquid, purity: >96%
Remarks:	Stock solutions prepared in nanograde Acetone. Water quality parameters (pH, temperature, dissolved oxygen content) monitored throughout test. Test fish challenged with Antimycin A. Data reported at 95% confidence level.
Reference:	Monsanto ABC 31116, Analytical Bio-Chemistry Labs, 1983

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]**; open-system **[ ]**; closed-system **[** X **]**
Species:	Daphnia magna
Exposure period:	48 Hours
Results:	EC_{50} (24h) = 0.51 mg/l

	EC_{50} (48h) = 0.20 mg/l
	NOEC = 0.10 mg/l
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:	Santoflex 14 purple liquid, purity: >95%
Remarks:	Stock solutions prepared in nanograde Acetone. Water quality parameters (pH, temperature, dissolved oxygen content) monitored throughout test. Data reported at 95% confidence level.
Reference:	Monsanto ABC 30688, Analytical Bio-Chemistry Labs, 1983

***4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae**

Species:	Selenastrum capricurnutum (freshwater alga)
Endpoint:	Biomass **[X]**; Growth rate **[X]**; Other **[ ]**
Exposure period:	96 Hours
Results:	EC_{50} (24h) = 1.9 ppm
	EC50 (96h) = 0.7 ppm
	NOEC = 0.3 ppm
	LOEC = 0.6 ppm
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	EPA Selastrum capricornutum Printz Algal Assay Test (1978) open-system **[ ]**; closed-system **[X]**
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:.	Santoflex 14 reddish purple gel , purity: >95%
Remarks:	Stock solutions prepared in reagent grade DMF. Concentrations determined by range-finding test. Confirmation of effect by in vivo chlorophyll a and cell numbers. Data reported at 95% confidence level.
Reference:	Monsanto BP-81-5-82 EG&G Bionomics, 1981

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rats, Sprague-Dawley Albino
Value:	2100 mg/kg b.w. Discriminating dose: 2510 mg/kg/bw
Method:	Defined Lethal Dose
GLP:	Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance:	CP-26658 Lots KC06-14 and KC06-17, purity: >95%
Remarks:	Five groups of male and female rats were fed a single oral dose of the undilated test article via oral gavage. Dosages were 1260, 1580, 2000, 2510 and 3160 mg/kg. Clinical signs of toxicity included reduced activity and appetite for 2-4 days for survivors, and increasing weakness, collapse and death for decedents in 1-4 days. Gross autopsy findings on decedents were hemorragic areas in the lungs, discolored livers and acute gastrointestinal inflammation. Survivors were sacrificed after seven days. All viscera of survivors appeared normal.
Reference:	Monsanto Y-73-169 Younger Laboratories, 1973

5.1.2 ACUTE INHALATION TOXICITY

Type:	LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[X]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain:	Rats, Sprague-Dawley Albino
Exposure time:	6 Hours
Value:	>0.14 mg/kg
Method:	Acute Inhalation
GLP:	Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance:	CP-26658 liquid, purity: >95%
Remarks:	A group of four rats was exposed to the test article at a concentration of 0.14 mg/l in warm (76.5°F) air for 6 hours. All animals survived. No clinical signs of toxicity were noted.
Reference:	Monsanto Y-67-101, Younger Laboratories, 1967

5.1.3 ACUTE DERMAL TOXICITY

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rabbits, New Zealand Albino
Value:	>5010 mg/kg b.w.
Method:	Defined Lethal Dose
GLP:	Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance:	CP-26658 Lots KC06-14 and KC06-17, purity: >95%
Remarks:	The undiluted test article was applied to the shaved skin of two groups of male and female rabbits at dose levels of 5010 and 7940 mg/kg/bw. Clinical signs of toxicity noted were reduced appetite and activity for 4-7 days in survivors, and increased weakness, collapse and death at 8 days for decedents. Gross autopsy findings in decedents included hemorragic areas in the lung, liver and spleen, and discoloration of the kidneys. General gastrointestinal inflammation was also noted. Survivors were

	sacrificed after 14 days. All viscera in survivors appeared normal.
Reference:	Monsanto Y-73-169 Younger Laboratories, 1973

***5.4 REPEATED DOSE TOXICITY**

Species/strain: Rats, Sprague-Dawley Albino
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral/Dietary
Exposure period: One Month
Frequency of treatment: Daily
Post exposure observation period:
Dose: 0, 500, 750, 1500 and 300 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle**[X]**; Historical **[ ]**
NOEL: 500 ppm
LOEL: Not Determined
Results: The test article was administered to groups of 25 male and 25 female rats in a controlled study for one month. Verification of test article stability and dose levels was verified via gas chromatography. Animals were observed twice daily and weighed weekly. Overall averages for dietary concentrations were established as 0, 450, 660, 1300 and 2800 ppm. There were no mortalities during the in-life portion of the study. Toxicity during the in-life phase was indicated by a dose-related reduction of food intake and reduced body weight gains in both males and females at all dietary levels. There were no clinical signs of toxicity observed during the study. There were no gross pathology changes noted at sacrifice which were considered treatment-related, and no significant differences in liver weights or organ coloration. The NOEL for male rats was considered to be 500 ppm. The same NOEL was marginally established for female rats, even though there was a slight, but not statistically significant difference seen in average body weights.
Method: Dunnett, C.W., A Multiple Comparison Procedure for Comparing Several Treatments with a Control, Jour. Am. Stat. Assoc. 50: 1096-1121, 1955
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex 14 dark liquid, Lot# KJ08-09, purity: >95%
Reference: Monsanto ML-87-309, Environmental Health Lab, 1987

***5.5 GENETIC TOXICITY IN VITRO**

A. BACTERIAL TEST

Type: Bacterial Reverse Mutation Assay - Ames
System of testing: Salmonella typhimurium TA-1535 TA-1537 TA-1538 TA-98 TA-100; Saccharomyces cerevisiae D4
Concentration: 0.001, 0.01, 0.1, 1.0 and 5.0 microliters/plate
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:
Cytotoxicity conc: With metabolic activation: 5.0 ul/plate (TA-98 only)
Without metabolic activation: 5.0 ul/plate (TA-98 only)
Precipitation conc: Not Determined
Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[X]**
Without metabolic activation:	**[ ]**	**[ ]**	**[X]**

Method: Ames Plate Test (Overlay method) 1975; OECD 471 equivalent

GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:	Santoflex 14 dark liquid, purity: >95%
Remarks:	The test article, in DMSO solvent, was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The test compound did not demonstrate mutagenic activity in any of the assasy conducted and was not considered to be mutagenic under test conditions.
Reference:	Monsanto BIO-76-229, Litton Bionetics, 1976

B. NON-BACTERIAL IN VITRO TEST

Type:	Forward Mutation Mouse Lymphoma Assay
System of testing:	L5178Y Mouse Lymphoma Cells
Concentration:	0.625 – 10.0 nl/ml without activation 1.25 – 60.0 nl/ml with activation
Metabolic activation:	With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:	
Cytotoxicity conc:	With metabolic activation: 60 nl/ml Without metabolic activation: 20 nl/ml
Precipitation conc:	Not Determined
Genotoxic effects:	+ ? - With metabolic activation: **[ ] [ ] [X]** Without metabolic activation: **[ ] [ ] [X]**
Method:	Clive, D., and Spector, J.F.S., Laboratory Procedure for Assessing Specific Locus Mutations at the TK Locus in Cultured L5178Y Mouse Lymphoma Cells. Mutation Res., 31:17-29, 1975
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:.	Santoflex 14 dark liquid, purity: >95%
Remarks:	The test compound in DMSO solution was evaluated for ability to increase mutations at the TK locus in mouse lymphoma cells at dose ranges of 0.625 to 10 nl/ml without activation and at 1.25 to 60 nl/ml with activation. Dose levels were established during a preliminary range-finding study. The dose levels selected included highly toxic treatments. Even at the highly toxic doses, the mutant frequency was comparable to negative controls. The test substance was considered to be inactive under assay conditions.
Reference:	Monsanto BO-78-225, Litton Bionetics, 1979

Type:	Forward Mutation Assay, CHO/HGPRT
System of testing:	Chinese Hamster Ovary cells
Concentration:	1-10 ug/ml without activation 10-30 ug/ml with activation
Metabolic activation:	With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:	
Cytotoxicity conc:	With metabolic activation: 7 ug/ml Without metabolic activation: 5 ug/ml
Precipitation conc:	Not Determined
Genotoxic effects:	+ ? - With metabolic activation: **[ ] [ ] [X]** Without metabolic activation: **[ ] [ ] [X]**
Method:	CHO/HGPRT Mutation Assay (1981) Hsie, et.al.
GLP:	Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:.	Santoflex 14 liquid Lot# KJ08-09, purity: >95%
Remarks:	The mutagenic potential of Santoflex 14 was tested in cultured Chinese hamster ovary (CHO) cells. Mutation at the Hypoxanthine guanine phosphororibosyl transferase (HGPRT) locus was measured. Dosages for the test article, dissolved in Acetone, were established with a range-finding experiment. No

Chemical-related mutagenicity was observed in either the initial or the confirmation experiment, with or without S9 activation, were noted. Santoflex 14 was not mutagenic in CHO cells under any test conditions.

Reference: Monsanto ML-87-340, Environmental Health Labs, 1988

Type: In vitro Cytogenetics Study
System of testing: Chinese Hamster Ovary (CHO) cells
Concentration: 1.5 – 15.0 ug/ml
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:
Cytotoxicity conc: With metabolic activation: 12.5 ug/ml
Without metabolic activation: 12.5 ug/ml
Precipitation conc: Not Determined
Genotoxic effects:

	+	?	-
With metabolic activation:	**[X]**	**[ ]**	**[ ]**
Without metabolic activation:	**[X]**	**[ ]**	**[ ]**

Method: Preston, Et. al., Mammalian In vivo and In vitro Cytogenics Assays: A report to the U.S. Gene-Tox Program (1981)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance:. Santoflex 14 opaque liquid #T870091, purity: >95%
Remarks: Treatment solutions were made using Acetone. Two range-Finding experiments were run to determine the optimum dose concentrations. MMS and CP were used as concurrent positive controls for treatment with and without S9 activation, respectively. Duplicate samples per treatment condition were used. Scoring for cytogenetic damage was performed on the solvent controls, positive controls, and the three highest dose levels of the test chemical. The cells were scored for both mitotic index and average cell generation time and compared to the solvent control. Average cell generation time was 12 hours for both, with a mitotic index of 5-8% Statistically significant increases in number of cells with structural aberrations and average structural aberrations/cell were observed at the 15 ug/ml level for the 48 hour harvest time and for average structural aberrations/cell at the 24 hour harvest time without S9 activation. A significant dose-response was not observed. The aberrant cells harvested at 24 and 48 hours included mainly cells with chromatid- and chromosome-type deletions, with a few decentrics and cells with chromatid interchanges. This was also observed in the solvent control. The positive MMS control yielded significant increases in both cells with structural aberrations and number of aberrations/cell. With S9 activation, a statistically significant increase in the number of cells with structural aberrations, and number of aberrations/cell was observed at the 10 ug/ml dose level, and for the number of aberrations/cell at 7.5 ug/ml and 12 hour harvest time. No dose-related response was observed. Aberrations were mainly deletions, with a few cells having chromatid interchanges, intrachanges and triradials. The positive control yielded the expected positive response. A retest confirmed results. Santoflex 14 was concluded to have a weak

clastogenicity in CHO cells under test conditions

Reference: Monsanto ML-87-341, Environmental Health Labs, 1989

*** 5.6 GENETIC TOXICITY IN VIVO**

Type: Mammalian Bone Marrow Metaphase Assay

Species/strain: Rats, Sprague-Dawley

Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**

Route of Administration: Oral gavage

Exposure period: 6, 18 and 30 hours

Doses: 1100 mg/kg/bw (slightly above ½ the oral LD50)

Results:

Effect on mitotic index or P/N ratio:

Genotoxic effects: + ? -
[ ] [ ] [X]

Method: Preston, Et. al., Mammalian In vivo and In vitro Cytogenics Assays: A report to the U.S. Gene-Tox Program (1981)

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance: Santoflex 14 dark oil, purity: >95%

Remarks: Groups of 5 male and female rats were dosed with 1050, 1100, 1200, 1500 and 2000 mg/kg/bw in two range-finding studies. Based upon the results, a dose level of 1100 mg/kg/bw was chosen as close to the maximum tolerated dose for the metaphase analysis. During the In vivo phase, test animals were observed for pharmacotoxicity immediately after dosing, and at 6, 18 and 30 hours. Observations indicated moderate to severe pharmacotoxic signs. Two to three hours prior to sacrifice, each animal received a single intraperitoneal dose of colchicine at 4 mg/kg/bw to arrest dividing cells in metaphase. Both femurs were removed from each animal after sacrifice. The distal end was snipped off one bone and the proximal end off the other. Bone marrow cells were flushed, washed and centrifuged, and slides were prepared using freshly prepared fixative. A total of 500 well-spread metaphase cells with a minimum of overlapping chromosomes were scored for the presence of chromosome aberration per experimental treatment point (50 per animal) by two investigators (25 each per animal). Cells judged acceptable for analysis based on cell morphology and total chromosome number were further analyzed with 100x oil immersion objective where abnormalities were detected and classified. The mean number of aberrations per cell per animal was analyzed for statistically significant increases by one-tailed t tests for each time interval. Santoflex 14 did not produce significant increases in the number of aberrations or in the number of aberrant metaphases at any of the three sacrifice times evaluated. Pharmacotoxic signs observed during the study indicated that the test chemical was dosed near the maximum tolerated dose. Conclusion was that the test chemical was negative in ability to induce structural chromosomal aberrations to the hemopoietic cells of the rat bone marrow under test conditions.

Reference: Monsanto PK-88-342, Pharmakon Research, 1988

***5.8 TOXICITY TO REPRODUCTION**

Type: Fertility **[]**; One-generation study **[]**; Two-generation study **[]**; Other **[ ]**

Species/strain:

Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**

Route of Administration:

Exposure period:

Frequency of treatment:

Post exposure observation period:

Premating exposure period: male: . . . . . , female:

Duration of the test:

Doses:

Control group: Yes **[ ]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**

NOEL Parental:

NOEL F1 Offspring:

NOEL F2 Offspring:

Results: General parental toxicity:
Toxicity to offspring:

Method:

GLP: Yes **[ ]** No **[ ]** ? **[ ]**

Test substance: . . . . . , purity: . . . . . .

Remarks:

Reference:

***5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY**

Species/strain:
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: .
Duration of the test:
Exposure period:
Frequency of treatment:
Doses:
Control group: Yes **[ ]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Maternal Toxicity:
NOEL teratogenicity :
Results:
Maternal general toxicity:
Pregnancy/litter data:
Foetal data:
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance: . . . . . , purity: . . . . . .
Remarks:
Reference:

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

Type: Immunotoxicity – Repeat Insult Patch Test
Human skin, Santoflex 14 Antiozonant
Shelansky Method (Procedings of the Toilet Goods Association, No. 19, May 1953)
Results: Fifty human volunteers not previously exposed to test rubber formulations were selected. Squares soaked in the test material were applied to the arm or back and held in place with tape. Patches were removed after 24 hours and the sites examined for reactions, after which the material was reapplied. Fifteen such primary applications were made, followed by a 2-week rest period. A challenge application was then applied as before, and to the same site. No reactions were produced by either the primary or challenge applications. There was no evidence of primary irritation or skin fatigue. There was no evidence of skin sensitization under the test conditions.
Remarks: Concentration of test article was not noted. Both male and female volunteers were used in the study.
Reference: Monsanto SH-65-3, Industrial Biology Labs, 1965

Type: Immunotoxicity – Repeat Insult Patch Test
Human skin, Unvulcanized Rubber containing Santoflex 14 Antiozonant
Shelansky Method (Procedings of the Toilet Goods Association, No. 19, May 1953)
Results: Fifty one human volunteers not previously exposed to test rubber formulations were selected. The test material, in the form of 1”

squares of unvulcanized rubber, was affixed to the upper arm of each test subject and covered with gauze (occluded). Patches were removed after 24 hours and the sites examined for reactions. Direct effects by single contact were graded with a numerical score ranging from 0 (no response) to 4 (severe response) for primary irritation. Choice of contact site for the second and all subsequent applications was based on the condition of the skin at the original contact site. If irritation occurred, a different site was chosen. If no irritation occurred, the test patch was reapplied to the same site. There were 15 such applications in the induction phase of the study. Following a 14-day rest period, a challenge application was applied at the original contact site. No visible skin changes were noted on any test subject during either the induction phase or the challenge phase of the study. The test article was considered to be negative for primary skin irritation, negative for skin fatigue by sequential contact, and negative for delayed contact hypersensitivity.

Remarks: Concentration of test article in the rubber compound was 3 parts per 100 parts of SBR 1000 rubber (3 phr) Both males and females were used in the study.

Reference: Monsanto SH-67-13, Industrial Biology Labs, 1967

B. Toxicodynamics, toxicokinetics

Type:

Results:

Remarks:

References:

*** 5.11 EXPERIENCE WITH HUMAN EXPOSURE**

Results: .
Remarks: .....
Reference: .....

6. REFERENCES

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12. Monsanto BP-81-5-82, Toxicity of Santoflex 14 to the Freshwater Alga Selenastrum capricornutum, EG&G Bionomics, April, 1981
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21. Monsanto SH-65-3, Repeated Insult Patch Test, Industrial Biology Laboratories, Inc. May 24, 1965
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I U C L I D

D a t a S e t

Existing Chemical	ID: 15233-47-3
CAS No.	15233-47-3
TSCA Name	1,4-benzenediamine, N-(1-methylheptyl)-N'-phenyl-
EINECS No.	239-281-1
Molecular Weight	296
Producer Related Part	
Company:	
Creation date:	08-NOV-2001
Substance Related Part	
Company:	
Creation date:	08-NOV-2001
Memo:	RAPA PPD Category
Printing date:	09-NOV-2001
Revision date:	
Date of last Update:	09-NOV-2001
Number of Pages:	19
Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 OECD and Company Information

Type: lead organisation
Name: American Chemistry Council (formerly Chemical Manufacturers Association) Rubber and Plastics Additives (RAPA) HPV Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States
Phone: 703-741-5600
Telefax: 703-741-6091

08-NOV-2001

Type: cooperating company
Name: Bayer Corporation
Country: United States

08-NOV-2001

Type: cooperating company
Name: Ciba Specialty Chemicals Corporation
Country: United States

08-NOV-2001

Type: cooperating company
Name: Crompton Corporation
Country: United States

08-NOV-2001

Type: cooperating company
Name: Flexsys America L.P.
Country: United States

08-NOV-2001

Type: cooperating company
Name: Noveon, Inc (formerly BF Goodrich)
Country: United States

08-NOV-2001

Type: cooperating company
Name: R.T. Vanderbilt Company, Inc.
Country: United States

08-NOV-2001

Type: cooperating company
Name: The Goodyear Tire & Rubber Company
Country: United States

08-NOV-2001

Type: cooperating company
Name: The Lubrizol Corporation
Country: United States

08-NOV-2001

Type: cooperating company
Name: UOP, LLC.
Country: United States

08-NOV-2001

1.0.2 Location of Production Site
-

1.0.3 Identity of Recipients
-

1.1 General Substance Information

Substance type: organic
Physical status: liquid
Purity: > 95 % w/w
08-NOV-2001

1.1.0 Details on Template
-

1.1.1 Spectra
-

1.2 Synonyms

N-phenyl - N'-(1-methylhepyl)-p-phenylenediamine
08-NOV-2001

UOP 688 Antiozonant
08-NOV-2001

1.3 Impurities
-

1.4 Additives
-

1.5 Quantity
-

1.6.1 Labelling
-

1.6.2 Classification
-

1.7 Use Pattern
-

1.7.1 Technology Production/Use
-

1.8 Occupational Exposure Limit Values
-

1.9 Source of Exposure
-

1.10.1 Recommendations/Precautionary Measures
-

1.10.2 Emergency Measures
-

1.11 Packaging
-

1.12 Possib. of Rendering Subst. Harmless
-

1.13 Statements Concerning Waste
-

1.14.1 Water Pollution
-

1.14.2 Major Accident Hazards
-

1.14.3 Air Pollution
-

1.15 Additional Remarks
-

1.16 Last Literature Search
-

1.17 Reviews
-

1.18 Listings e.g. Chemical Inventories
-

2.1 Melting Point

Value:
Remark: Unknown, no studies available
08-NOV-2001

2.2 Boiling Point

Value: 431 degree C at 1013 hPa
Method: other: no data
GLP: no
08-NOV-2001 (1)

2.3 Density

Type: relative density
Value: 1.003 at 15.6 degree C
Method: other: no data
GLP: no
Result: Specific gravity = 1.003
08-NOV-2001 (1)

2.3.1 Granulometry
-

2.4 Vapour Pressure

Value:
Remark: Unknown, no studies available
08-NOV-2001

2.5 Partition Coefficient

log Pow:
Method: OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year:
Result: Method not applicable.
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
08-NOV-2001 (2)

2.6.1 Water Solubility

Qualitative: not soluble
Method: OECD Guide-line 105 "Water Solubility"
Remark: Evaluation as part of Certificate of Analysis
Result: Insoluble;
pH Value, pKa Value: Unknown, no studies available
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
08-NOV-2001 (2)

2.6.2 Surface Tension
-

2.7 Flash Point
-

2.8 Auto Flammability
-

2.9 Flammability
-

2.10 Explosive Properties
-

2.11 Oxidizing Properties

Result:
Remark: Unknown, no studies available
08-NOV-2001

2.12 Additional Remarks

Memo: Fat Solubility
Method: OECD 116
Result: 100%
08-NOV-2001 (2)

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000 molecule/cm3
Rate constant: .000000000229 cm3/(molecule * sec)
Degradation: 50 % after .6 hour(s)
Method: other (calculated): AOP Program (v1.89)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

3.1.2 Stability in Water
-

3.1.3 Stability in Soil
-

3.2 Monitoring Data (Environment)
-

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
Media: other: air - water - soil - sediment
Air (Level I):
Water (Level I):
Soil (Level I):
Biota (L.II/III):
Soil (L.II/III):
Method: other: EPIWIN, Level III Fugacity Model
Year: 1999
Result:

Media	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	0.0248	1.12	1000	7.34e-013
Water	8.94	900	1000	2.61e-014
Soil	43.4	900	1000	3.56e-016
Sediment	47.6	3.6e+003	0	1.76e-014

Media	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	615	9.94	20.5	0.331
Water	275	358	9.18	11.9
Soil	1.34e+003	0	44.6	0
Sediment	367	38.1	12.2	1.27

Persistence Time: 1.33e+003 hr

Reaction Time: 1.54e+003 hr
Advection Time: 9.86e+003 hr
Percent Reacted: 86.5
Percent Advected: 13.5
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

3.3.2 Distribution
-

3.4 Mode of Degradation in Actual Use
-

3.5 Biodegradation
-

3.6 BOD5, COD or BOD5/COD Ratio
-

3.7 Bioaccumulation
-

3.8 Additional Remarks
-

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other
Species: other: Freshwater fish
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: .067
Method: other: ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: Chemical may not be soluble enough to measure this predicted effect.
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

Type: other
Species: other: Saltwater fish
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: .094
Method: other: ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: Chemical may not be soluble enough to measure this predicted effect.
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: other
Species: Daphnia sp. (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : .093
Method: other: ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: Chemical may not be soluble enough to measure this predicted effect.
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

Type: other
Species: Mysidopsis bahia (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : .00134
Method: other: ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Reliability: (2) valid with restrictions
Acceted calculation method
08-NOV-2001 (3)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Endpoint:
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: .072
Method: other: ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: Chemical may not be soluble enough to measure this predicted effect.
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

4.4 Toxicity to Microorganisms e.g. Bacteria
-

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish
-

4.5.2 Chronic Toxicity to Aquatic Invertebrates
-

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms
-

4.6.2 Toxicity to Terrestrial Plants
-

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species
-

4.7 Biological Effects Monitoring
-

4.8 Biotransformation and Kinetics
-

4.9 Additional Remarks
-

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type:	LD50
Species:	rat
Strain:	other: Holtzman
Sex:	male
Number of Animals:	5
Vehicle:	other: corn oil
Value:	4.3 mg/kg bw
Method:	other: Method described by Weil, C.S., Biometrics 8, 249, 1952
Year:	1952 GLP: no
Test substance:	other TS: Commercial product, >95% purity
Method:	UOP 688 was administered orally to six groups , each composed of 5 male albino rats, weight range 219-251 grams. Each dose was administered either undiluted or as a 10% volume/volume solution in corn (Mazola) oil. Dosage levels tested were 0.046, 0.10, 2.15, 4.46, 10.0, and 21.5 mg/kg body weight. All animals were observed closely for gross signs of systemic toxicity and mortality during the day of dosage, and at least once daily thereafter for 14 days. All animals were subject to gross necropsy at study termination.
Result:	Animals in the 0.046, 0.1, and 2.15 mg/kg dosage levels generally exhibited normal appearance and behaviour throughout the 14 day period. Rats at the 4.64 mg/kg dose level began showing depression, slowed righting reflexes, and diarrhea on the second day following dosage. On the fourth day after dosage, one rat showed labored respiration, ataxia, depressed righting, placement, and pain reflexes, and a marked bloody nasal discharge. These signs generally continued until death occurred, or until the fifth day following dosage when the two surviving rats appeared normal. The rats in the 10.0 and 21.5 mg/kg doe levels showed diarrhea, unkempt fur, depression, depressed relexes, and a dark oily stain in the perineal area on the day after dosage. These signs continued until death occurred. Death was preceded by lacrimation and coma.
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag:	Critical study for SIDS endpoint

08-NOV-2001 (4)

5.1.2 Acute Inhalation Toxicity

Type:
Species:
Strain:
Sex:
Number of
Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: GLP:
Test substance:
Remark: Unknown, no studies available.
Not an appropriate route of exposure due high boiling point.
08-NOV-2001

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: New Zealand white
Sex: male/female
Number of
Animals: 10
Vehicle:
Value: > 2000 mg/kg bw
Method: other: U.S. Code of Federal Regulations 40 CFR 163
Year: GLP:
Test substance: other TS: Commercial product, >95% purity
Method: The test material was applied to five male and five female white New Zealand white rabbits. The dose was applied to the abdominal skin which had been previously been shaven. The abdominal skin area of all the rabbits was abraded by making a series of longitudinal minor epidermal incisions placed two to three centimeters apart, using a hypodermic needle as a cutting tool. The abrasions were sufficiently deep to penetrate the epidermis, but not to induce bleeding. The undiluted sample was applied at a dosage level of 2.0 grams/kg of body weight. The test sample was kept in contact with the skin on at least 10% of the body surface. During the exposure period, each rabbit was observed for signs of toxicity at two, four and five and one half hours post application. After 23 ¾ to 24 hours of skin contact exposure, any unabsorbed sample remaining on the skin was removed by gentle sponging with a moistened towel. Rabbits were observed for 14 days following completion of the exposure period. Examinations for gross signs of systemic toxicity were carried out twice daily during this period. At the end of the 14 day observation period, rabbits were weighted, sacrificed and gross necropsy was performed.
Remark: study reviewed by lab QA Director
Result: One female rabbit was found dead on day two. Necropsy

revealed diarrhea stains around the anus, congested lungs, a mottled and darkened liver, stomach and intestine which appeared autolytic and pale but congested kidneys. Erythemia and edema followed by desquamation and atonia were seen at the application site in all surviving animals. Four rabbits exhibited spotted whitening on the day of exposure completion. Systemic effects were limited to transient nasal discharge in two animals and transient green colored urine in one animal.
Reliability: (1) valid without restriction
Meets National standards method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (5)

5.1.4 Acute Toxicity, other Routes
-

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration:

Exposure: Semiocclusive
Exposure Time: 24 hour(s)
Number of Animals: 6
PDII: 1.5
Result:
EC classificat.:
Method: other: U.S. Code of Federal Regulations 40 CRF 163
Year: GLP:
Test substance: other TS: Commercial product, >95% purity
Method: 0.5 ml undiluted test material was applied under one inch square surgical gauze patches to two abraded skin areas and two intact skin areas on each of six New Zealand White rabbits. After 24 hours of skin contact exposure, any unabsorbed sample remaining on the skin was removed by gentle sponging with a moistened towel. The reactions were scored immediately after removal of the patches (24 hour reading), and again two days later (72 hour reading).
Remark: study reviewed by lab QA Director
Result: Irritative effects noted during the course of the study included very slight to well defined erythema, at the abraded and intact sites of all animals. Very slight to slight edema scores were noted in five animals on the abraded and intact sites. The Primary Irritation Index was found to be 1.5. Some loss of skin resiliency (atonicity) was noted. No evidence of corrosivity was observed.
Reliability: (1) valid without restriction
Meets National standards method
09-NOV-2001 (5)

Species: rabbit
Concentration: undiluted

Exposure: Semiocclusive
Exposure Time:
Number of
Animals: 6
PDII:
Result:
EC classificat.:
Method: other: U.S. Code of Federal Regulations 49 CFR 173.136 -137
Year: 1992 GLP: yes
Test substance: other TS: Commercial product, Lot #0483, >95% purity
Method: The primary dermal irritation/corrosivity potential was evaluated when applied to the skin of 3 male and 3 female rabbits under 3 minute, 1 hour, and 4 hour semi-occluded conditions. Each application site was examined for erythemia and edema according to the Draize method.
Result: No evidence of corrosion was observed at any of the test sites for any of the exposure periods.

Not considered corrosive to the skin of rabbits
Reliability: (1) valid without restriction
GLP Guideline study
09-NOV-2001 (6)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time:
Comment: other: see method
Number of
Animals: 9
Result:
EC classificat.:
Method: other: U.S. Code of Federal Regulations 40 CFR 163
Year: GLP:
Test substance: other TS: Commercial product, >95% purity
Method: 0.1 ml of the undiluted test material was applied to the left or right eye of each of nine rabbits. The opposite eye served as a control. The treated eyes of six rabbits were left unrinsed. The treated eye of three rabbits were rinsed after 30 seconds for 60 seconds with 200 ml of lukewarm water. Examinations for gross signs of eye irritation were made approximately 24, 43, and 70 ½ hours and four, seven, ten, thirteen, sixteen, and nineteen days following application. Scoring of irritative effects was according to the method of Draize.
Remark: study reviewed by lab QA Director
Result: Non-rinsed eyes - Irritative effects noted during the study included isolated occurrences of mild corneal opacity with up to one-quarter of the corneal area involved in the two

rabbits. Conjuctival effects included isolated occurrences of mild erythema in five rabbits. Total irritation score ranged from 0-5.

Rinsed eyes - Mild corneal irritation was observed in the rinsed eye group. These effects generally cleared after four days post-treatment with opacity occurring once after this reading in one rabbit. Sporadic occurrences of mild to moderate conjunctival irritation on days 13 and 19 were noted in three rabbits. The total irritation scores ranged from 0-7.

09-NOV-2001 (5)

5.3 Sensitization

Type:	Patch-Test
Species:	human
Number of Animals:	15
Vehicle:	other: acetone
Result:	not sensitizing
Classification:	not sensitizing
Method:	other: Adapted from the repeated insult patch test procedure described by Draize (Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, pp. 52-55, The Association of Food and Drug Officials of the United States, 1959)
Year:	GLP: no
Test substance:	other TS: Commercial product
Method:	0.1 ml of a 20% acetone solution of the sample (equivalent to 20 mg of the test material) was applied to a ¾ x 7/8 inch piece of filter paper. After the acetone had evaporated, the filter paper was place on the skin of 15 human subjects. Nine patch applications were made to the same location on the upper arm over a period of two weeks. A challenge patch was applied to skin area not previously exposed to the test material.
Result:	None of the 15 subjects tested exhibited any evidence of sensitization.

09-NOV-2001 (7)

5.4 Repeated Dose Toxicity

-

5.5 Genetic Toxicity 'in Vitro'

Type:	Ames test
System of testing:	Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100
Concentration:	0.0005, 0/001, 0.0025, 0.005, 0.01, 0.05, 0.1, 0.5 ug/plate
Cytotoxic Conc.:	Without metabolic activation: >0.07 ug/plate; Precipitation conc: 0.59 ug/plate
Metabolic activation:	with and without
Result:	negative
Method:	other: Ames Salmonella/Microsome Plate Test, Protocol 401, Edition 14
Year:	GLP: yes
Test substance:	other TS: Commercial product, purity >95%
Remark:	Examination of mutagenic activity in the presence and absence of liver microsomal preparations was conducted. Solvent control (dimethyl sulfoxide) and specific positive control compounds were assayed concurrently with the test material. The concurrent solvent control data were used as a basis for evaluating results.
Result:	The test material did not exhibit genetic activity in any of the assays conducted and was not mutagenic to the S. typhimurium indicator organism under the test conditions.
Reliability:	(1) valid without restriction GLP Guideline study
Flag:	Critical study for SIDS endpoint

09-NOV-2001 (8)

5.6 Genetic Toxicity 'in Vivo'

-

5.7 Carcinogenicity

-

5.8 Toxicity to Reproduction

-

5.9 Developmental Toxicity/Teratogenicity

-

5.10 Other Relevant Information

-

5.11 Experience with Human Exposure

-

(1) From internal technical bulletin, 1981

(2) Evaluation as part of Certificate of Analysis, by Fine Pharmaceutical Laboratories, Ltd., Hamilton, Ontario, Canada, January 24, 2001

(3) Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

(4) Unpublished study, "Acute Oral Adminstration of UOP 604 and UOP 688 to Rats", Hill Top Research Institute, Inc. Miamiville, OH, February 13, 1963

(5) Unpublished study, "Acute Dermal Toxicity, Primary Skin Irritation and Acute Eye Irritation Potential of UOP 688", Hill Top Research, Inc., Cincinnati, OH, September 22, 1981

(6) Unpublished study, "Skin Corrosivity Study of UOP 688 in Rabbits (DOT/UN Regulations)", Hazelton Wisconsin, Inc, Madison WI, June 25, 1993.

(7) Unpublished study, "Repeated Insult Patch Test of UOP 688 and 12267", Hill Top Research, Inc., September 20, 1962.

(8) Unpublished study, "Mutagenicity Test on XPA-28-86/UOP 688 in the Ames Salmonella/Micorsomal Reverse Mutation Assay", Hazelton Laboratories America, Inc., Kensington, MD, October 13, 1981.

7.1 End Point Summary
-

7.2 Hazard Summary
-

7.3 Risk Assessment
-

I U C L I D

D a t a S e t

Existing Chemical	ID: 68953-84-4
CAS No.	68953-84-4
EINECS Name	N,N'-diaryl-p-phenylenediamines
EINECS No.	273-227-8

Producer Related Part	
Company:	Goodyear Chemicals Europe
Creation date:	06-APR-1998
Substance Related Part	
Company:	Goodyear Chemicals Europe
Creation date:	06-APR-1998

Printing date:	30-OCT-2001
Revision date:	
Date of last Update:	20-FEB-2001

Number of Pages:	28

Chapter (profile):	Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.1, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8, 5.9
Reliability (profile):	Reliability: 1, 2
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

Date: 30-OCT-2001
2. Physico-chemical Data ID: 68953-84-4

2.1 Melting Point

Value: 90 - 105 degree C
Decomposition: ambiguous
Method: other: ASTM D-1519
Year: 1993
GLP: no
Reliability: (2) valid with restrictions
Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.
31-JUL-2000 (34)

2.2 Boiling Point
-

2.4 Vapour Pressure
-

2.5 Partition Coefficient

log Pow: 3.4 - 4.3
Method: OECD Guide-line 117 "Partition Coefficient (n-octanol/water), HPLC Method"
Year: 1995
GLP: yes
Remark: The product exhibits much lower values than DDT (6.2) which provides a benchmark for highly bioaccumulative chemicals.
The test substance contains 3 major components.
Result: # Methyl Groups -0 log Pow 3.37
Methyl Groups -1 log Pow 3.82
Methyl Groups -2 log Pow 4.28

The major components of the test substance displayed partion coefficients between 3.4 and 4.3.
Reliability: (1) valid without restriction
01-AUG-2000 (28)

log Pow: > 3.7 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: for N,N'-Diphenyl-p-phenylenediamine
Reliability: (1) valid without restriction

20-FEB-2001 (9)

log Pow: > 4.3 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: For N-phenyl-N'-(o-tolyl)-p-phenylenediamine
Reliability: (1) valid without restriction
31-JUL-2000 (9)

log Pow: > 4.6 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: For N,N'-Di(o-tolyl)-p-phenylenediamine
Reliability: (1) valid without restriction
20-FEB-2001 (9)

2.6.1 Water Solubility
-

3.1.1 Photodegradation
-

3.1.2 Stability in Water

Type:
Method:
Year: 1994 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Remark: See Biodegradation Studies
Reliability: (1) valid without restriction
31-JUL-2000

3.3.1 Transport between Environmental Compartments
-

3.5 Biodegradation

Type: anaerobic
Inoculum: activated sludge, domestic
Concentration: 100 mg/l related to Test substance
Degradation: .64 % after 28 day
Result: other: not readily biodegradable
Method: OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year: 1994 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Reliability: (1) valid without restriction
31-JUL-2000 (22)

Type: anaerobic
Inoculum: activated sludge
Degradation: 0 % after 28 day
Method: other: OECD 301 Manometric Respirometry, modified according to

EEC Round RobinTest "Assessment of Respirometry" DGX 1/283/82 Rev. 6, EEC Directive 79/831, Annex V, Part C
Year: 1990 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Reliability: (1) valid without restriction
31-JUL-2000 (6)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: flow through
Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 14 day
Unit: mg/l Analytical monitoring: yes
NOEC: .28
LC50: .43
Method: OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"
Year: 1996 GLP: yes
Test substance: other TS
Method: Test water was generated by adding the test substance in acetone to a larger volume of water which was stirred, allowed to settle, and then siphoned to a stock solution holding tank. This stock solution was then metered into exposure tanks for the fish experiments. A range-finding trial exposed carp to to nominal levels of 2.5, 5, 10, and 25 mg/L (ppm) of the test substance. Survival rates were up to 80% within the first 48 hours for the three (3) highest dose levels and the 2.5 mg/L induced no mortality in the first 48 hours although 90% deaths were seen through Day six (6).

In the definitive phase, duplicate test tanks contained 10 carp each and the test substance nominal concentrations of 0, 0.1, 0.23, 0.51, 1.1, and 2.5 mg/L (ppm). Chemical analysis (HPLC) of the test substance in the test tanks on Days -0, -3, -7, and -14 showed that mean concentrations for the 14-day test period were 0.053, 0.12, 0.19, 0.28, and

0.67 mg/L (ppm). Fish densities were 0.35 g biomass/L flowing test solution per day. Tank volume turnover for the flow-through system was 6.5/day. Carp were monitored daily for mortality and signs of erratic swimming behavior for 14 days during exposure. Body weights and lengths were recorded for representative fish prior to study initation, and on all test fish on Day 14. A LC50 value was then calculated.

Result: Carp died only at the highest test substance concentration; 2/20 on Day-3, 7/20 on Day-7, and 20/20 by Day-14. Other findings at the 0.67 mg/L (ppm) level included darkened pigmentation on the fish (likely due to adsorption of the test chemical), lethargic swimming behavior, and loss of equilibrum. There were no test substance-related effects on body lengths or weights.

Test substance: Tested as the commercial product

Reliability: (1) valid without restriction

20-FEB-2001 (29)

Type: flow through

Species: Oncorhynchus mykiss (Fish, fresh water)

Exposure period: 14 day

Unit: mg/l Analytical monitoring: yes

NOEC: .14

LC50: .26

Method: OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"

Year: 1997 GLP: yes

Test substance: as prescribed by 1.1 - 1.4

Method: Test water was generated by adding the test substance in acetone to a larger volume of water which was stirred, allowed to settle, and then siphoned to a stock solution holding tank. This stock solution was then metered into exposure tanks for fish experiments. A preliminary study in trout was performed using nominal concentrations of the test substance of 0.1, 0.23, 0.51, 1.1, and 2.5 mg/L. Mortality rates were 100% at the highest level by Day-3, and was 80% by Day-7 at 1.1 mg/L.

In the definitive phase, duplicate test tanks contained 10 trout each, Test substance nominal concentrations of 0, 0.094, 0.19, 0.38, 0.75, and 1.5 mg/L (ppm) were chosen. Chemical analysis (HPLC) of the test substance in the test tanks on Days -0, -7 and -14 showed that mean concentrations for the 14-day test period were 0.062, 0.093, 0.14, 0.35, and 0.66 mg/L (ppm). Fish densities were 0.079 g biomass/L

flowing test solution per day. Tank volume turnover for the flow-through system was 6.5/day. Fish were monitored daily for mortality and signs of erratic swimming behavior for 14-days during exposure. Body weights and lengths were recorded for representative fish prior to study initiation, and on all test fish on Day-14. LC50 values were calculated for 96-hours and 14-days.

Result: Fish died only at 0.35 and 0.66 mg/L concentrations; 0/20 and 1/20 died by Day-2 and 1/20 and 19/20 by Day -4 , respectively. Further, 100 % of the high dose (0.66 mg/L) fish died by Day-5 and 17/20 of the 0.37 mg/L fish by Day-14. Other findings at the two highest levels included darkened pigmentation of the fish, lethargic swimming behavior, and loss of equilibrium. There were test substance-related effects on 14-day body lengths and weights in the 0.35 mg/L group. The calculated LC50 for the test substance in the study at 96-hours was 0.48 mg/L and 0.26 mg/L at 14-days. The No Observed Effect Concentration (NOEC) was 0.14 mg/L at 96-hours and 14-days.

Reliability: (1) valid without restriction

31-JUL-2000 (37)

4.2 Acute Toxicity to Aquatic Invertebrates

Type:
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: yes
NOEC: .36
EC50: 1.8
Method: OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year: 1996 GLP: yes
Test substance: other TS
Method: A range-finding study used ten (10) 24-hour old daphnids exposed to nominal levels of 0, 13,22,36,60, and 100 mg/L of the test substance. Immpbilization (15%) of the daphnids occurred at the highest level (100 mg/L). Sublethal lethargy was observed at all but the lowest test concentration (13 mg/L). Brown matter, apparently the test substance since brown precipitate was observed in the media, was observed to adhere to both surviving and non-surviving daphnids.

In the definitive phase, duplicate aquaria containing 10

daphnids each and test substance nominal concentrations of 0, 1.3, 2.2, 3.6, 6.0 and 10 mg/L (ppm) were prepared. Mean values for the test substance concentrations in the test media were determined by averaging chemical analyses (HLPC) of 0-hours and 48-hours.

Daphnia immobilization and aquaria observations were made at 24- and 48-hours following the study initation. From these data, an Effective Concentration in one-half the organisims (EC50) and a No Observed Effect Concentration (NOEC) were estimated.

Result: Measured concentrations of the test substance ranged from 19 to 29% of nominal levels. At the highest concentration (1.8 mg/L), 25 % of the daphnids were immobilized at 48-hours of exposure. For the 0.68 and 1.1 mg/L groups, Five (5) % of the daphnids were immobile. No immobilization was observed at 0.20 and 0.36 mg/L exposures. Lethargic activity was not observed at any treatment level. Brown particulates, perhaps the test substance, were observed to adhere to the test daphnids, with some buoyed to the surface of the aquaria by this particulate material. The results indicated that the EC50 for the test substance was 1.8 mg/L. The No Observed Effect Concentration (NOEC) was shown to be 0.36 mg/L.

Test substance: Tested as the commercial product

Reliability: (1) valid without restriction

31-JUL-2000 (27)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Selenastrum capricornutum (Algae)
Endpoint: biomass
Exposure period: 72 hour(s)
Unit: µg/l Analytical monitoring: yes
NOEC: 4.3
EC10: 4.3
EC50: 18
Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 1996 GLP: yes
Test substance: other TS
Method: A range-finding trial used nominal levels of 0, 1,10, 100, and 1000 ug/L (ppb) of the test substance and a solvent control in algae cultures (approximately 1x104 cells per flask). Following 72-hours incubation, algal cell densities were determined using a hemacytometer. Values were

127,76,109,69 and 1%, respectively, of the solvent control response. These values were used to set exposures for the definitive phase.

In the definitive phase, triplicate algal cultures were exposed to the test substance at nominal concentrations of 16, 31, 63,130, 250, and 500 ug/L (ppb). Cell densities were monitored at 24-, 48-, and 72-hours following study initiation. From these data, EC50 (50% decrease) values for Biomass (EbC50) and Growth Rate (ErC50) were calculated. Test substance concentrations in the test media were determined at 0- and 72-hours using HLPC. The mean concentrations were 7.5, 13, 14, 28, 50, and 79 ug/L (ppb).

Result: The inhibitions of algae Growth Rates for the test substance in the definitive 72-hour study were 0, 2, 15, 20, 32, and 38% (relative to pooled control values) for the measured test substance concentrations of 7.5, 13, 14, 28, 50, and 79 ug/L (ppb). Corresponding inhibitions of Biomass generation were 15, 41, 59, 63, 81, and 91%. Individual cell appearances were found microscopically to be normal for surviving cells except cellular bloating was noted at the highest exposure level. Calculations indicated that the ErC50 for the test substance was > 79 ug/L (ppb) while the EbC50 was 18 ug/L (ppb). The No Observed Effect Concentrations (NOECs) were assumed to be equivalent to EC10 values, and accordingly were EbC10 = 4.3 ug/L (ppb) and ErC10= 31 ug/L (ppb).

The EC50 values for the test substance ranged from 18 to > 79 ug/l (ppb) for Biomass increases and Growth Rates. The NOECs ranged from 4.3 to 31 ug/L (ppb) for these parameters.

Test substance: Tested as the commercial product

Reliability: (1) valid without restriction

31-JUL-2000 (30)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: µg/l Analytical monitoring: yes
NOEC: 31
EC10: 31
EC50: > 79
Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 1996 GLP: yes
Test substance: other TS
Method: A range-finding trial used nominal levels of 0, 1,10, 100, and 1000 ug/L (ppb) of the test substance and a solvent

control in algae cultures (approximately 1x104 cells per flask). Following 72-hours incubation, algal cell densities were determined using a hemacytometer. Values were 127,76,109,69 and 1%, respectively, of the solvent control response. These values were used to set exposures for the definitive phase.

In the definitive phase, triplicate algal cultures were exposed to the test substance at nominal concentrations of 16, 31, 63,130, 250, and 500 ug/L (ppb). Cell densities were monitored at 24-, 48-, and 72-hours following study initiation. From these data, EC50 (50% decrease) values for Biomass (EbC50) and Growth Rate (ErC50) were calculated. Test substance concentrations in the test media were determined at 0- and 72-hours using HLPC. The mean concentrations were 7.5, 13, 14, 28, 50, and 79 ug/L (ppb).

Result: The inhibitions of algae Growth Rates for the test substance in the definitive 72-hour study were 0, 2, 15, 20, 32, and 38% (relative to pooled control values) for the measured test substance concentrations of 7.5, 13, 14, 28, 50, and 79 ug/L (ppb). Corresponding inhibitions of Biomass generation were 15, 41, 59, 63, 81, and 91%. Individual cell appearances were found microscopically to be normal for surviving cells except cellular bloating was noted at the highest exposure level. Calculations indicated that the ErC50 for the test substance was > 79 ug/L (ppb) while the EbC50 was 18 ug/L (ppb). The No Observed Effect Concentrations (NOECs) were assumed to be equivalent to EC10 values, and accordingly were EbC10 = 4.3 ug/L (ppb) and ErC10= 31 ug/L (ppb).

The EC50 values for the test substance ranged from 18 to > 79 ug/l (ppb) for Biomass increases and Growth Rates. The NOECs ranged from 4.3 to 31 ug/L (ppb) for these parameters.

Test substance: Tested as the commercial product

Reliability: (1) valid without restriction

31-JUL-2000 (30)

5. Toxicity

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50

Species: rat

Strain:

Sex: no data
Number of Animals:
Vehicle:
Value: > 2000 mg/kg bw
Method: other: Directive 84/49/EEC, B.1
Year: 1990 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Reliability: (1) valid without restriction
01-AUG-2000 (7)

Type: LD50
Species: rat
Strain:
Sex: male/female
Number of Animals: 10
Vehicle: other: corn oil
Value: > 5000 mg/kg bw
Method: other: US EPA 40CFR798.2650, Oral Toxicity-Limit Test
Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Method: Five (5) male and five (5) female young adult rats (Sprague-Dawley) were administered a single dose of the test substance by gavage. The test substance was dispersed in corn oil (Sigma Chemical Company) and administered at a dosage of 5000 mg/kg. The animals were observed for clinical signs of toxicity at approximately 1-, 4- and 24-hours following administrations on the day of dosing and daily thereafter for 14-days. Body weights were recorded on Day-0, Day-7 and Day-14. All animals were subjected to a gross necropsy at study termination.
Result: One (1) animal died during the 14-day observation period. Clinical signs observed included decreased activity, decreased muscle tone, and diarrhea. No significant impairment on body weight gains were noted in either the male or female rats. Necropsy of the animal that died during the study revealed discolored kidneys, spleen, and liver. No visible lesions were observed in any of the animals at terminal necropsy. The estimated acute oral LD50 (combined sexes) for the test substance was determined to be > 5000 mg/kg.
Reliability: (1) valid without restriction
01-AUG-2000 (20)

5.1.2 Acute Inhalation Toxicity
-

5.1.3 Acute Dermal Toxicity

Type:	LD50
Species:	rabbit
Strain:	
Sex:	male/female
Number of Animals:	10
Vehicle:	other
Value:	> 2000 mg/kg bw
Method:	OECD Guide-line 402 "Acute dermal Toxicity"
Year:	1995 GLP: yes
Test substance:	other TS
Method:	Albino rabbits (five males and five females) were shaved in the caudal portion of the animals' trunks. One (1) day later, a 2000 mg/kg dose of 40 mesh test substance (obtained by grinding in motar/pestle) was placed onto the skin sites (approximately 10% of the body surface areas). The application sites were then covered with gauze, plastic, and elastic wraps and finally secured with non-irritating tape. After 24-hours of skin contact to the exposure areas, the gauze patches were removed and adhering test substance removed with moistened gauze. Skin test sites were scored for signs of erythema (redness) and edema (swelling) according to Draize procedures from Day-1 to Day-14 following cessation of exposures. Animals were observed for adverse clinical signs, mortality, and body weights (Day-0, Day-7, and Day-14). Necropsies were performed on the final day of observations (Day-14).
Remark:	A limit test
Result:	The test substance induced no deaths or apparent adverse clinical signs. Mild irritation (Grades 1,2 erythema; Grade 1 edema) was seen at skin sites of treated rabbits for periods ranging fron Day-1 to Day-10. Staining of skin was noted due to the dark color of the test substance. A body weight decrease was seen in one (1) of the ten (10) rabbits between Day-7 and Day-14. No compound-related non-dermal findings were observed in the study. No mortality or adverse clinical/necropsy changes were observed associated with the test substance. The dermal LD50 for the test substance was shown to be > 2000 mg/kg.
Test substance:	Tested as the commercial product
Reliability:	(1) valid without restriction

01-AUG-2000 (26)

5.1.4 Acute Toxicity, other Routes

-

5.4 Repeated Dose Toxicity

Species:	rat	Sex:	male/female
Strain:	Fischer 344		
Route of admin.:	oral feed		
Exposure period:	28 days		
Frequency of treatment:	Daily		
Post. obs. period:	2 weeks		
Doses:	0, 7.5, 30 and 120 mg/kg/day		
Control Group:	yes, concurrent vehicle		
NOAEL:	7.5 mg/kg		
LOAEL:	30 mg/kg		
Method:	other: Oral 4-week dietary study		
Year:	1996	GLP:	yes
Test substance:	as prescribed by 1.1 - 1.4		

Method: The test substance was prepared by grinding in a coffee mill, sieved through a 125 um mesh screen and mixed with rodent diet NIH-07 at 0, 120, 470, 1900 ppm (0, 7.5, 30, and 120 mg/kg/day). Stability, homogenity, and dose verification were performed to confirm compliance with protocol. The prepared dosed feed was presented to 14 male and 14 female rats (Fischer 344) per test group at twelve weeks of age for four (4) weeks. Six (6) rats/sex/group were held for post-exposure in two (2) week recovery groups. Test rats were monitored for body weights, feed consumption, and clinical signs. Collections were performed on six (6) or three (3) rats/sex/group at 28-days and 42-days sacrifice periods for blood (hematologies and clincal chemistries) and urinalyses, respectively. Necropsies were performed on all rats, and organs were weighed (liver,kidneys, pituitary, uteri, heart, brain, spleen, thryoids, adrenals, testes, and ovaries). These and other major organs were preserved in formalin, stained with H&E, and subjected to microscopic evaluations. Liver, kidney, and urinary bladder slices were subjected to immunohistochemical staining for proliferating cell nuclear antigen (PCNA) for assessment of cellular division.

Result: The test substance was shown to be completely stable in diets for 46-days. Mixing procedures produced homogeneous diets that were found within 10% of target concentrations. No compound-related deaths occurred, The boby weights were not affected in male rats whereas the high dose female rats displayed 5% body weight decreases during study weeks two (2) through four (4). Food consumption was decreased in the high dose males and in the mid- and high dose females mainly during study weeks two (2) through four (4). Various test substance-induced hematological changes occurred that included: increased mean corpuscular volumes and decreased mean corpuscular hemoglobin concentrations (high dose males and females) and blood bilirubin and cholesterol increases (high dose males and females). Most blood endpoints tended to approach control levels during week two (2) of the recovery

period. No dose-related urinary changes were seen. Organ

weight increases were seen at 28-days for liver and kidneys (high dose males and females; mid-dose females) and heart and spleen (high dose females). Only the kidney weights did not reach control levels by 42-days. There were no gross tissue or microscopic changes related to the test substance. Proliferating cell nuclear antigen (PCNA) exams showed cell division changes for: increases for liver cells (High dose males and females and mid-dose males at 28-days only); changes for kidney cells (decreases in high dose females at 28-days and increases in high dose males and females at 42-days; and increasing trend in urothelial cells in bladder (low and mid-dose males and females at 28-days). Macrocytic anemia was the primary change in rats related to the test substance administration. This change was reversible within 2 weeks following dietary exposure as were liver weight and serum cholesterol elevations. These changes were very minor, and had no apparent toxicological significance in this study. The lack of dose-responsiveness in the PCNA data provides results of uncertain importance to the assessment of the toxicity of this test substance.

Reliability: (1) valid without restriction
02-AUG-2000 (11)

Species: rat Sex: male/female
Strain: other: Fischer 344/N TacfBR
Route of admin.: gavage
Exposure period: 21 days
Frequency of treatment: Daily
Post. obs. period:
Doses: 0, 0.1, 0.3, 1.0, and 3.0 g/kg/bw
Control Group: yes, concurrent vehicle
LOAEL: 100 mg/kg bw
Method: other: Oral 3-Week Range-Finding Study
Year: 1994 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Remark: A 4-week diet-study was also conducted.
Result: Doses of 1.0 and 3.0 g/kg/day of WINGSTAY 100 administered by gavage for up to 6 days were lethal for male and female F344 rats. The only pertinent gross finding of all unscheduled deaths was the paleness of most external surfaces and viscera. The mid-low (0.3 g/kg/day) and low (0.1 g/kg/day) doses caused time and dose related significant body weight gain loss, liver weight increase and hepatocellular labeling index increase at 0.1 g/kg. Therefore, in the subchronic studies, the recommended daily dose of WINGSTAY 100 should not exceed 100 mg/kg/day, if administered by gavage.

Test substance: The test substance was prepared in an olive oil suspension for dosing
Reliability: (1) valid without restriction
02-AUG-2000 (5)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Ames/E. coli preincubation; Salmonella typhimurium TA-98, 100, 1535, 1537, 1538, and WP2 uvrA
Concentration: Salmonell stains without S9 activation:0.167, 0.5, 1.67, 5, 16.7, and 50 ug/plate; Salmonella strains with S9 activation:1.67, 5, 16.7, 50, 167, and 500 ug/plate; E.coli with/without S9 activation:1.67, 5, 16.7, 50, 167, and 500 ug/plate
Cytotoxic Conc.:
Metabolic activation: with and without
Result: positive
Method: other: Japan's Industrial Safety & Health Law, a combination of OECD Guidelines 471 and 472.
Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Method: In a preliminary assay, revertant frequencies for all doses of the test substance in tester strains TA1535, TA1537, TA98, TA100, and WP2 uvrA with S9 metabolic activation, and in tester strains TA1535, TA1538, TA98, TA100 and WP2 uvrA without S9 activation, approximated the concurrent negative controls. However, statistically significant, increases in revertant frequencies, to approximately 1.7- to 2.5-fold control values, were observed in tester strain TA1538 with S9 metabolic activation and in tester strain TA1537 without S9 metabolic activiation. In addition, the increases observed in strain TA1538 with S9 metabolic activation were dose dependent.

In a confirmatory assay, revertant frequencies for all doses of the test substance in tester strains TA1535, TA100, and WP2 uvrA with metabolic activation, and in tester strains TA1535, TA1538, TA98, TA100, and WP2 uvrA without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in revertant frequencies, to control values, were observed in tester strains TA1537, TA1538, and TA98 with metabolic activation. Statistically significant increases in revertant frequencies, to control values, also were observed in tester strain TA98 without S9 metabolic activation. However, these latter increases apparently were not dose related.

The test substance was re-evaluated in all five Salmonella strains with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation. Revertant frequencies for all doses of the test substance in tester strains TA1535, TA1537, and TA100 with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation, approximated or were less than control values. Statistically significant, dose-dependent increases in revertant frequencies, to control values, were observed in tester strains TA1538 and TA98 with S9 metabolic activation. All

positive and negative control values in all assays were within acceptable limits.

Result: The test substance was shown to cause mutations in Ames/Salmonella strains TA1538 and TA98 with S9 activation.

Reliability: (1) valid without restriction

04-AUG-2000 (16)

Type: Ames test

System of testing: Ames/Salmonella-E.coli Liquid Pre-incubation Assay in Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 and in E.coli strain WP2 uvrA.

Concentration: Salmonella strains with S9: 1.67, 5, 16.7, 50, 167, and 500 ug/plate; Salmonella strains without S9: 0.167, 0.5, 1.67, 5, 16.7, and 50 ug/plate; E.coli with/without S9: 1.67, 5, 16.7, 50, 167, and 500 ug/ plate.

Cytotoxic Conc.:

Metabolic activation: with and without

Result: positive

Method: other: Japan's Industrial Safety & Health Law, a combination of OECD Guidelines 471 and 472.

Year: 1994 GLP: yes

Test substance: as prescribed by 1.1 - 1.4

Method: In a preliminary assay, revertant frequencies for all doses of the test substance in tester strains TA1535, TA1537, TA100, and WP2 uvrA with and without S9 metabolic activation approximated the concurrent negative controls. However, statistically significant, increases in revertant frequencies, to control values, were observed in tester strains TA1538 and TA98 with S9 metabolic activation. In addition, the increases observed in strain TA1538 with S9 metabolic activation were dose dependent.

In a confirmatory assay, revertant frequencies for all doses of the test substance in tester strains TA1535, TA100, and WP2 uvrA with metabolic activation, and in tester strains TA1535, TA1537, TA1538, TA100, and WP2 uvrA without S9 metabolic activation, approximated control values.

Statistically significant, dose-dependent increases in revertant frequencies,to control values, were observed in tester strains TA1537, TA1538, and TA98 with metabolic activation. Statistically significant increases in revertant frequencies, to control values, also were observed in tester strain TA98 without S9 metabolic activation. However, these latter increases apparently were not dose related.

The test substance was re-evaluated in all five Salmonella strains with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation. Revertant frequencies for all doses of the test substance in tester strains TA1535, and TA100 with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in revertant frequencies, to

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control values, were observed in tester strains TA1537, TA1538, and TA98 with S9 metabolic activation. All positive and negative control values in all assays were within acceptable limits.

Result: The test substance was shown to cause mutations in Ames/Salmonella strains TA1537, TA1538 and TA98 with S9 metabolic activation.

Reliability: (1) valid without restriction

04-AUG-2000 (17)

Type: Cytogenetic assay
System of testing: Chromosomal aberration assay in CHO cells
Concentration: 0.4, 2, 4, and 25 ug/mL
Cytotoxic Conc.:
Metabolic activation: with and without
Result: negative
Method: OECD Guide-line 473 "Genetic Toxicology: In vitro Mammalian Cytogenetic Test"
Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Method: In the structural Chromosomal Aberration assay, duplicate cultures were established for each dose level. Three treatment schedules were used: a) First set of cultures were treated for 5-hours with the appropriate dose of the test sample in Ham's F12 serum free (F12SF) medium either in the presence or absence of S9 metabolic activation along with concurrent negative and positive controls followed by three (3) Puck's saline washes and medium replacement; b) Second set of cultures were treated for 24-hours with the test substance or control articles in Ham's F12 medium containing five (5) % serum (F12FCM5%) without S9 metabolic activation, and; c) Third set of cultures were treated for 48-hours with

the test substance or control articles in F12FCM5% medium without S9 metabolic activation. Two (2) to three (3) hours prior to harvest, Colcemid (2X10-7M) was added to all sets of cell cultures to arrest dividing cells in metaphase. CHO cells were harvested at the eppropriate time and metaphase slides were prepared and stained.

The data from one hundred metaphases from each culture (200 metaphases per dose point) were pooled for statistical analysis. Data were evaluated by using the chi-square of aberrant versus normal cells while comparing each dose level to its concurrent negative control. The data were also analyzed for statistical significance by pairwise t-tests comparing the number of aberrations per cell in each treated dose versus the negative control.

Result: Analysis of the data for the 24-hour treatment with the test substance indicated that there were statistically significant dose-related increases in the frequency of aberrations/cell and proportion of aberrant metaphases at doses 2 and 4 ug/mL. The data for the 2 and 4 ug/mL doses produced a statistically significant linear trend when

analyzed by the Cochran/Armitage Linear Trend Test. To verify the biological sighificance of this finding, the 24-hour treatment was repeated.

In the confirmatory assay, the test substance was re-evaluated at doses of 25 ug/mL with S9 metabloic activation (5-hour treatment) and 0.4, 2, and 4 ug/mL without S9 metabolic activation (24-hour treatment). Analysis of the data for the 5-hour treatment did not produce statistically significant increases in aberrations/cell or in proportion of aberrant metaphases.

Analysis of the data for the 24-hour treatment indicated a statistically significant increase in aberrations/metaphase at the mid-dose (2 ug/mL) with S9 metabolic activation but there were no significant increases in the proportion of aberrant metaphases. However, when the data for 2 ug/mL (0.045 + or - 0.208) were compared to the untreated control data (0.025 + or - 0.157) or to Pharmakon historical acetone data (0.034 + or - 0.021), there were no statistically significant increases in the frequency of aberrations/metaphase. Therefore, the positive finding in the t-test for 2 ug/mL was considered a statistically artifact with no biological significance. There were no other statistically significant increases in aberration/metaphase or in the proportion of aberrant metaphases at any of the remaining dose levels for the 24-hour treatment.

The test substance was judged negative (non-clastogenic) based on its inability to reproducibly induce dose-related increases in structural chromosomal aberrations in CHO cells.
Reliability: (1) valid without restriction
20-FEB-2001 (19)

Type: DNA damage and repair assay
System of testing: E. coli Pol A1- Liquid Suspension Assay
Concentration:
Cytotoxic Conc.:
Metabolic activation: without
Result: positive
Method: other
Year: 1980 GLP: no
Test substance: as prescribed by 1.1 - 1.4
Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000 (32)

Type: other: Transformation Assay
System of testing: Balb/3T3 In Vitro Transformation Assay
Concentration: .01 ug/ml to 1.0 ug/ml
Cytotoxic Conc.:
Metabolic activation: without
Result: negative
Method: other
Year: 1981 GLP: no
Test substance: as prescribed by 1.1 - 1.4
Reliability: (2) valid with restrictions
Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.
04-AUG-2000 (12)

Type: other: Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes
System of testing: Hepatocytes form male Fischer 344 (F344/Crl) rats
Concentration: Slightly above their limits of solubility
Cytotoxic Conc.:
Metabolic

activation: without
Result: negative
Method: other: Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes on Test substance Condensation Products
Year: 1999 GLP: yes
Test substance: other TS: Test substance condensation products with Dicylopentadiene
Method: The test substance, 1,4-Benzenediamine, N.N'-mixed Ph and tolyl. derivs., was reacted with Dicyclopentadiene in varying ratios, resulting in three condensation products. Each of these condensation products were subjected to independent in vitro unscheduled DNA synthesis (UDS) assays with hepatocytes form male Fischer 344 (F344/Crl) rats. All three (3) condensation products were tested at concentrations slightly above their limits of solubility in the tissue culture medium. Hepatocytes were exposed to test substances for 18-20 hours to allow bioactivation and DNA repair. The assay was based on the incorporation of 3H-thymidine into the hepatocyte's DNA during repair of DNA-damage. This incorporation was monitored by counting Net Nuclear Grains (NNG) formed on photographic emulsion placed on the cells adhering to glass slides. Criteria for a positive response included : (a) Significant increase in number of grains at two (2) levels of exposure above negative control levels, (b) A dose-responsiveness in grain counts up to toxic levels of exposure, and (c) At least one (1) value for NNG that is five (5) or above. A negative response is reported for NNG's that are <0, and an equivocal or inconclusive response are results that are 0<#<5.
Result: In all the Unscheduled DNA Synthesis Assay (UDS) trials, the three (3) negative controls {the untreated cells control, F,

and Dimethylsulfoxide (DMSO)} had negative values for Net Nuclear Gain (NNG) counts (<0). A positive control, 2-Aminofluorene (2-AF) was positive for induction of UDS; the mean NNG counts were 45.92 and 58.99 in the first and second assays, respectively, indicating assay validity. (i.e., hepatocytes were capable of metabolic activation and DNA repair). The positive control responses occurred at toxic levels. UDS assay results for NNGs were in the range of -26 to -46, demonstrating a lack of UDS activity for the three (3) condensation products at concentrations greater than their solubilites in the test media. The results indicated that, under controlled laboratory conditions, the condensation products from the reaction of 1.4-Benzenediamine, N,N', mixed Ph and tolyl. derivs. with Dicyclopentadiene were negative for induction of UDS in rat hepatocytes at concentrations up to and greater than their solubilities. This assay demonstrated a lack of genetic activity in this mammalian DNA-repair test system.
Reliability: (1) valid without restriction

07-AUG-2000 (36)

5.6 Genetic Toxicity 'in Vivo'

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex:
Strain:
Route of admin.: oral feed
Exposure period: 24 hours
Doses: 50 ug/ml and 10 ug/ml
Result: negative
Method: other: Drosophila melanogaster (Fruit Fly) System
Year: 1979 GLP: no
Test substance: as prescribed by 1.1 - 1.4
Result: Negative under conditions of the assay
Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000 (31)

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex:
Strain:
Route of admin.: oral feed
Exposure period: 24 hours
Doses: 0.05 mg/ml and 0.63 mg/ml
Result: negative
Method: other: Drosophilia SLRL Assay
Year: 1979 GLP: no
Test substance: as prescribed by 1.1 - 1.4
Result: Negative under conditions of the assay.
Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound

procedure for that time period and the study was properly conducted.

04-AUG-2000 (13)

Type: Micronucleus assay
Species: mouse Sex: male/female
Strain: CD-1
Route of admin.: i.p.
Exposure period: single dosing
Doses: 0, 250, 1250, 2500 mg/kg test chemial; 0.5 g/kg TEM (+ control)
Result: negative
Method: OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Method: Nine (9) groups of mice (CD-1) were acclimated to laboratory conditions for 25-days prior to initiation of the study. THe mice were randomized by body weight and assigned to groups using a computer-generated random number list.

Each group of mice was comprised of ten (10) animals(five (5) males/five (5) females). Each mouse received a single intrperitoneal dose at 10 mL/kg of body weight. The test substance at dose levels of 250, 1250, anf 2500 mg/kg was adnimistered to three (3) groups of mice which were sacrificed at 24-, 48-, and 72-hours post dose. Concurrently, the negative control, Dimethylsulfoxide (DMSO)/corn oil, was administered, as dose volume of 10 mL/kg of body weight, to three (3) groups of mice. A group of these mice were included in each sampling time. The positive control, Triethylenemelamine at 0.5 mg/kg, was administered to one (1) group of mice and sacrificed at 24-hours post dose.

All mice were sacrificed and their femurs were removed. Their bone marrow was removed by flushing. Smears were made of the suspended cells.

One (1) thousand young erythrocytes were evaluated for a change of ratio of polychromatic erythrocytes (PCE) to normochromatic cells (NCE).

5. Toxicity

Result: There were no statistically significant depressions in the PCE/NCE ratios in any groups of mice except for the 2500 mg/kg group at 48-hours sacrifice time ($p<0.01$) which was an indication that the test substance had reached the bone marrow and was toxic to erythrocytes.

Analysis of the micronucleus data for the groups treated with the test substance indicated that there were no statistically significant increases in the frequency of

micronucleated PCEs. The test substance was judged negative (non-clastogenic) based on its inability to induce micronucleated PCEs.
Reliability: (1) valid without restriction
04-AUG-2000 (18)

Type: other: 32P Postlabeling Assay for Detection of Adduct Formation in Rat DNA
Species: rat Sex: male/female
Strain: other: Fischer 344/N TacfBR
Route of admin.: gavage
Exposure period: 7 days
Doses: 0., 0.3, 1.0, and 3.0 g/kg/bw
Result: negative
Method: other: 32P Post-Labeling Assay for DNA Adduct Formtion
Year: 1995 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Remark: The purpose of the study was to determine the potential of WINGSTAY 100 to bind covalently to liver and urinary bladder DNA of male and female rats after in vivo administration of WINGSTAY 100.
Result: Under conditions of the study, the test substance did not induce DNA-adducts in the liver and urinary bladder DNA of rats.
Reliability: (1) valid without restriction
07-AUG-2000 (4)

5.8 Toxicity to Reproduction

Type: Two generation study
Species: rat Sex: male/female

Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure Period: F0 exposed during 10 weeks premating, 2 weeks of mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 males and females exposed for 10 weeks prior to mating.
Frequency of treatment: Daily
Premating Exposure Period
male: 10 weeks
female: 10 weeks
Duration of test: 9 months
Doses: 0, 120, 400 or 1500 ppm.
Control Group: yes, concurrent no treatment
Method: OECD Guide-line 416 "Two-generation Reproduction Toxicity Study"
Year: 2000 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Method: This study was designed in compliance with EPA GLP and USEPA FIFRA guidelines. Dose levels were established from a rangefinding study at Research Triangle Institute which employed dietary levels of 120, 1900, and 5700 ppm of WINGSTAY 100. The top level was lethal to dams and offspring, 1900 ppm induced one nonviable litter in 9 total, and thus, the top dose for the definitive study was decreased by 20% to assure high viability in test group. No effects were seen at 120 ppm.

This study used 30 SpragueDawley rats/sex/dose (F0) exposed to diets containing 0, 120, 400 or 1500 ppm WINGSTAY 100 during 10 weeks premating, 2 weeks mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 litters were culled to 10 each each at 4 days postnatal (PND) 30 other F1 males and females/group chosen for pairing, and fed WINGSTAY 100 as above for 10 weeks prior to mating. After mating/gestation of F1, the resulting F2 rats were delivered, and maintained through weaning period (to PND 21). Weekly body weights (BWs) and food consumption (FC), and daily clinical observations were recorded. Necropsies and histopathology (primary kidneys) were performed on selected rats from each sex/group/generation (all F0 and F1 dams at PND21, three F1 and F2 pups/test group at PND21). Remaining F1 and F2 rats were euthanized without examination. Data were collected on vaginal cytology, mating, pregnancy, litter, and pup parameters.
Remark: WINGSTAY 100 induced dystocia (difficult deliveries) in pregnant rats which may have led to prolonged gestation and increased perinatal deaths, decreased live births, and increased pup weights. In addition, polycystic lesions were observed at all dose levels. Prolonged gestation has previously been associated with the WINGSTAY component DPPD, and polycystic kidneys were observed in DPamine-treated

rats. Based upon adult toxicities, reproductive and offspring endpoints, there was no NOEL for WINGSTAY 100 in this study.

Result: High dose females had decreased Body Weights (BWs) relative to other test groups throughout majority of study period. Mortality during gestation/lacation were: F0 dams- 0 in 24 pregnancies, 0/27, 3/24, 4/25; F1- 0/22, 0/23, 1/22, 1/24. Numbers of pregnancies with no live births: F0- 0, 1, 1, 10; F1- 0, 1, 1, 2. Gestational length: F0- 22.2 days, 22.4 days, 22.8*, 23.5*; F1- 22.2, 22.8*, 23.1*, 23.2* (* = statistically sighnificant). The number of live pups/litter: F0-15.6, 14.1, 11.9, 7.6*; F1- 15.6, 13.7, 13.3, 10.8*. Pups weights (g) on PND 0: F0- 6.38, 6.79*, 6.93*, 6.63*; F1- 6.32, 6.89*, 6.99*, 6.63*. WINGSTAY 100-related kidney lesions were observed grossly (as white or clear cysts) and microscopically (polycyctic findings with variable severity): F0 adults- males 0/0, 0/0, 0/0, 0/1 and females 0/0, 0/0, 0/2, 3/9; F1 weanlings- males 0/23, 1/25, 8/20, 10/11 and females 0/22, 5/26, 7/18, 11/11; F1 adults- males 0/30, 5/30, 10/30, 21/30 and females 0/30, 2/30, 1/30, 18/30; F2 weanlings- males 0/60, 3/64, 6/19, 15/16 and females 0/60, 5/64, 8/19, 15/15. The severity of kidney lesions were also dose related.

Reliability: (1) valid without restriction

11-FEB-2001 (35)

5.9 Developmental Toxicity/Teratogenicity

Species: rat Sex: female
Strain: Sprague-Dawley
Route of admin.: gavage
Exposure period: 10 days
Frequency of treatment: Dosed on days 6-15 gestation
Duration of test:
Doses: 0, 20, 70, 200 mg test material in 5 ml corn oil/kg
Control Group: yes, concurrent vehicle
NOAEL Maternalt.: 70 mg/kg bw
NOAEL Teratogen.: <= 200 mg/kg bw
Method: OECD Guide-line 414 "Teratogenicity"
Year: 1995 GLP: yes
Test substance: other TS
Method: Preliminary trials in 8 rats/group indicated that 600 mg/kg was lethal to 50% of maternal rats while 200 mg.kg caused decreased body weights in materanl and fetal animals. There were no effects at 20 or 70 mg/kg. Consequently, 200 mg/kg was selected as the top (high) dose in the definitive study, Confirmation of the test dose solutions were confirmed analytically.

The definitive study used 25 inseminated female rats per test group (0, 20, 70, and 200 mg of test substance/kg doses in five (5) mL corn oil/kg). The animals were dosed on Days 6-15 gestation. Body weights, food consumption, liver

weights, clinical changes, pregnancy rates, and corpora lutea counts were followed along with numerous fetal parameters. All fetuses were weighed, sexed, and assessed for external and visceral abnormalities. One (1) half of the fetuses were examined for skeletal abnormalities while the second half were subjected to cranial bone assessments.

Remark: Administered in 5 ml corn oil/kg by gavage

Result: The test substance induced no lethality. Deficits were seen in maternal body weights (Day-12 and body weight change from Day-6 to Day-15) and food consumption (during treatment period) at the highest dose only (200 mg/kg). Pregancy rates, litter sizes, number of live fetuses, uterine implantation, and all gestational parameters were unaffected by chemical treatment. There was a linear trend towards lower body weights in fetuses with increasing doses (approximately 5% decrease in 200 mg/kg group). Assessment of cranial, skeletal, visceral, and external appearance discerned no compound-related abnormalities (malformatiuons or variations) according to established criteria. The test material produced minimal effects (body weight) to maternal rats from oral dosing of 200 mg/kg during pregnancy. There was no induction by the test chemical of birth defects (major or minor) in fetal animals.

Test substance: Tested as the commercial product

Reliability: (1) valid without restriction

08-AUG-2000 (21)

Species: rat Sex: male/female

Strain: Sprague-Dawley

Route of admin.: oral feed

Exposure period: Varied, see method

Frequency of treatment: Varied, see method

Duration of test:

Doses: 2500 ppm

Control Group: yes, concurrent vehicle

Method: other: Mechanistic Study

Year: 2000 GLP: no

Test substance: as prescribed by 1.1 - 1.4

Method: The toxicity of the test substance to maternal and 1st. generation offspring was evaluated by exposing CD (Sprague-Dawley) rats to fixed dietary concentrations of 2500 ppm during different time periods (i.e. exposures during prebreed, mating, gestation, and/or lactation). Five (5) Groups (20/sex/Group) were studied including: Group one (1)- Negative control; Group two (2)- Dietary test substance during prebreed and mating, exposures ended on gestation day (gd)-0; Group three (3)- Dietary test substance during gestation and lactation, exposures began on gd-0; Group four (4)- Dietary test substance during prebreed, mating, gestation, and lactation, the Positive control and; Group

five (5)- Dietary test substance during prebreed, mating, gestation, and lactation, plus 600 ppm of iron gluconate in the drinking water for prebreed through lactation.

5. Toxicity

Males and females were paired within Groups (1:1) for the two-week mating period. Once a given female was found to be sperm positive {date designated as gestation day (gd)-0}, "her" male was euthanized and discarded. On the day of delivery (pnd-0), pups were counted, sexed, and weighed. On pnd-4, litters were culled to ten, counted, sexed, and weighed. On pnd-7, -14, and -21, pups were counted, sexed, and weighed. All pups were euthanized and one (1)/sex/litter necropsied on pnd-21. Dead pups on pnd-0 and -1 were examined macroscopically (necropsied) for polycystic kidneys. Female body weights and feed consumption were recorded weekly during prebreed, gestation, and postnatally. At necropsy on pnd-21, the maternal spleen, liver, and kidneys were weighed and retained in a fixative. Kidneys form Groups one (1) and five (5) were examined histopathologically. Blood sampling was performed on gestation day-21 and pnd-21 from all females (pregnant) by tail vein withdrawal. Blood sampling was performed on pnd-21 on the F1 offspring by withdrawal from the abdominal vena cava at sacrifice. The blood parameters assessed were: WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, Platelets, WBC Diffential (to correct the RBC and WBC counts for Nucleated Red Blood Cells) and Methemoglobin. On gd-21, a second sample of blood was taken via tail vein from all pregnant females in all Groups, with plasma frozen for possible subsequent analysis for specific hormones. For Group three (3), any female who had not yet delivered by gestation day-23 had blood taken from the tail vein and plasma frozen. On pnd-21, the spleen, liver, kidneys, and heart from one (1) pup/sex/litter were weighed and retained in a fixative. The kidneys from all offspring were examined histologically. Statistical analysis included both parametric and nonparametric tests for continuous and discrete data.

Remark: The objectives of this study were to confirm and further characterize prviously-observed effects following the test substance administration to pregnant rats. This study was designed (1) to determine the necessary and sufficient timing of exposure to maternal females at a fixed dietary concentration of the test substance to produce dystocia, prolonged gestation, and polycystic kidneys in offspring, (2) to determine whether the test substance results in demonstratable macrocytic anemia in maternal animals, (3) to determine if there is treatment-induced anemia and whether iron supplementation ameliorates or prevents the anemia, dystocia, and/or polycystic kidneys, and (4) to determine if F0 parental females exhibit polycystic kidneys due to

dietary exposure to the test substance.

Result: F0 Males: The test substance intake over the prebreed period (Study Days 0-28) averaged 180 mg/kg/day for all three (3) exposed Groups {two (2), four (4), and five (5)}. Iron gluconate intake in Group five (5) averaged 56 mg/kg/day (Study Days-0 to 28). Clinical observations were found to be unrelated to compound administration.

F0 Females: The test substance intake averaged 187-192

mg/kg/day for Groups two (2), four (4) and five (5) during gestation days (gd)-0 to 28. Iron gluconate intake during gestational days-0 to 28 in Group five (5) averaged 53 mg/kg/day. Clinical observations during gestation included one (1) female found dead in Groups three (3) and four (4), alopecia predominantly in Groups four (4) and five (5), pale eyes and tail, pale (not otherwise specified) almost exclusively in Groups three(3), four (4) and five (5) (all exposed), pilorection in Groups three (3), four (4) and five (5), and delayed parturition in Groups three (3), four (4), and five (5). The hematological profile of maternal rats on gestation day-21 found no evidence on macrocytic anemia in any groups.

REPRODUCTIVE/DEVELOPMENTAL: Gestational index (a measure of live litters relative to pregnant females) was significantly increased in Groups three (3) and four (4) but not in Group five (5). Male mating, fertility, and pregnancy indices were equivalent across all groups. Gestational length in days was significantly prolonged in Group three (3) (23.6+/-0.2), Group four (4) (23.8+/-0.2), and Group five (5) (23.5+/-0.2) relative to Control Group value (22.2+/-0.1) and the value in Group two (2) (22.3+/-0.1). Number of implantation sites per litter was significantly reduced in Group five (5). Percent of postimplantation loss was significantly increased in Groups three (3) and four (4). Pups per litter were significantly reduced in Groups three (3), four (4) and five (5), and number of dead pups per litter were significantly increased in Groups three (3) and four (4). Weanling gross and microscopic findings were limited to hydronephrosis in Groups one (1) and two (2), gas in intestines in Group two (2), and gross evidence of polycystic kidneys in Groups three (3), four (4), and five (5). Maternal hematologic profiles at sacrifice (21 days after delivery) indicated statistically significant changes in most erythrocyte parameters. The white blood cell differential counts indicated changes (as percent of cells examined) as follows: increase in segmented neutrophils and decrease in lymphocytes only in Group four (4), with no treatment-related changes in the percentages of monocytes or eosinophils. Histopathologic assessment was performed on

kidneys of all maternal rats in Groups one (1) and five (5). Polycystic kidneys were observed microscopically (but not macroscopically) in three (3) of 20 animals in Group five (5), with no polycystic kidneys observed in Group one (1).

The timing of exposure to the test substance with respect to pregnancy is an important determinant of toxicity. Exposure of F0 females to 2500 ppm of the test material during gestation is necessary and sufficient to produce dystocia (prolonged gestation). It is necessary and sufficient to expose F0 dams during gestation and/or lactation to produce polycystic kidneys in the F1 offspring. Since no Groups were exposed only during gestation or only during lactation, it is not possible to further define how exposure timing

affects this endpoint. There was no demonstratable macrocytic anemia in gestation day-21 (gd-21) F0 dams in any treatment Group, but at post delivery day-21 (pnd-21), F0 mothers exposed prior to and during mating, gestation, and lactation were anemic. The F1 offspring at pnd-21 did not consistently display evidence of macrocytic anemia. Iron supplementation did not affect pnd-21 maternal anemia, dystocia, or incidence/severity of polycystic kidneys in the F1 offspring. However, perinatal survival of the offspring was affected. Microscopic, but not macroscopic evidence of polycystic kidneys was found in 15 percent of dams treated prior to and during mating, gestation, and lactation (with iron supplementation). Controls had neither macroscopic nor microscopic indications of polycystic kidneys. Exposure of animals to the test substance prior to and during mating {Group two (2)} did not appear to result in adverse affects to offspring. Furthermore, exposure during the prebreed/mating periods did not increase the affects produced from gestation/lactation exposures only.

Reliability: (2) valid with restrictions
Although this study was not conducted to GLP, the test parameters used were based on a sound scientific design.

09-AUG-2000 (15)

(4) American Health Foundation, 32P Postlabeling Assay for Detection of Adduct Formation by WINGSTAY 100 and R-59 in Rat Liver and Urinary Bladder DNA, AHF Study No. 93-63 to The Goodyear Tire & Rubber Company, 1995

(5) American Health Foundation, Preliminary Oral 3-Week Range-Finding Study for Administration of WINGSTAY 100 and R-59 in Male and Female Fischer 344 Rats, AHF Study R-1626 to The Goodyear Tire & Rubber Company, 1994.

(6) Bayer AG Data

(7) Bayer AG, Report No. 19778, December 10, 1990.

(9) Bayer AG, Unpublished Data, July 2, 1992

(11) Four-Week Dietary Study of WINGSTAY 100 in Fischer 344 Rats, Report # AHF R1664, American Health Foundation, 1/31/96

(12) Litton Bionetics, Inc., Balb/3T3 In Vitro Transformation Assay of NAILAX, Genetics Assay No.5419 to The Goodyear Tire & Rubber Company, 1981.

(13) Litton Bionetics, Inc., Drosophilia SLRL Assay with Nailax to The Goodyear Tire & Rubber Company, 1979.

(15) Mechanistic Study of Wingstay 100, Report Study # RTI 65C-6429-500, Research Triangle Park, February 11, 2000

(16) Pharmakon USA, Report # Ph301-GY-001-93 to The Goodyear Tire & Rubber Company, 1993

(17) Pharmakon USA, Report # Ph301-GY-004-93 to The Goodyear Tire & Rubber Company, 1994

(18) Pharmakon USA, Report # Ph309-GY-001-93 to The Goodyear Tire & Rubber Company, 1993.

(19) Pharmakon USA, Report # Ph320-GY-001-93 to The Goodyear Tire & Rubber Company, 1993.

(20) Pharmakon USA, Report # Ph402-GY-001-93 to The Goodyear Tire & Rubber Company, 1993.

(21) Reseach Triangle Research, Developmental Toxicity Evaluation of WINGSTAY 100 Administered by Gavage to CD (Sprague-Dawley) Rats, Report # 65C-5962-100/200 to The Goodyear Tire & Rubber Company, July 11, 1995.

(22) Ricerca, Inc., Biodegradation Study of a Rubber Antioxidant, Document Number 6011-94-0037-BC-001 to The Goodyear Tire & Rubber Company, 1994

(26) Springborn Laboratories, An Acute Toxicity Study in Rabbits with WINGSTAY 100 (Limit Test), Report # S94-001-3097.29 to The Goodyear Tire & Rubber Company, August 24, 1995.

(27) Springborn Laboratories, WINGSTAY 100-Acute Toxicity to Daphnids Under Flow-Through Conditions, Report # 96-1-6328 to The Goodyear Tire & Rubber Company, June 26, 1996.

(28) Springborn Laboratories, WINGSTAY 100-Determination of n-Octanol/Water Partition Coefficient, Report # 95-9-6103 to The Goodyear Tire & Rubber Company, December 12, 1995

(29) Springborn Laboratories, WINGSTAY 100-Prolonged (14-day) Acute Toxicity to Common Carp Under Flow-Through Conditions, Report # 96-2-6362 to The Goodyear Tire & Rubber Company, June 28, 1996

(30) Springborn Laboratories, WINGSTAY 100-Toxicity to the Freshwater Green Alga, Report # 96-4-6454 to The Goodyear Tire & Rubber Company, July 2, 1996.

(31) The Goodyear Tire & Rubber Company, Biological Effects of Nailax B in a Drosophilia melanogaster (Fruit Fly) Test System, 1979.

(32) The Goodyear Tire & Rubber Company, DNA Damage by WINGSTAY

100 Lot 48-3012 in the E. coli Pol A1- Assay, 1980.

(34) The Goodyear Tire & Rubber Company, WINGSTAY 100, Material Safety Data Sheet, 1993

(35) Two-Generation Reproductive Toxicity Evaluation of WINGSTAY 100 Administered in the Feed to CD (Sprague-Dawley) Rats, Report #: 65C-6429-400/200, Research Triangle Institute, 12/8/00.

(36) Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes on Wingstay 100 Condensation Products RWC-7703, RWX-7704, and RWC-7706, American Health Foundation, December 20, 1999

(37) WINGSTAY 100-Prolonged (14-Day) Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) Under Flow-through Conditions, Report # 96-11-6700, Springborn Laboratories, 2/21/97.

101-72-4
p-Phenylenediamine, N-Isopropyl-N'-Phenyl-

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	75-80 °C
Decomposition:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Sublimation:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Method:	FF83.9-1 Initial and Final Melting Point of Organic Compounds 1996
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Remarks:	Capillary Method
Reference:	ASTM D-1519 / Flexsys Physical Methods of Analysis

*2.2 BOILING POINT

Value:	161 °C
Pressure:	at 1 mm Hg
Decomposition:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Method:	Not listed
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	
Reference:	Monsanto Toxicology Profile of Santoflex IP, 1990

†2.3 DENSITY (relative density)

Type:	Bulk density **[ ]**; Density **[X]**; Relative Density **[ ]**
Value:	1.180
Temperature:	20 °C
Method:	FF97.8-1 Flexsys Standard Method 1997
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Remarks:	Density of solids by displacement
Reference:	Flexsys Physical Methods of Analysis

*2.4 VAPOUR PRESSURE

Value:	0.00343 mm Hg
Temperature:	90 °C
Method:	calculated **[ ]**; measured **[X]**
	Not listed
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	
Reference:	Monsanto Toxicology Profile of Santoflex IP, 1990

*2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

Log Pow:	3.28 Log P
Temperature:	Not Determined
Method:	calculated **[X]**; measured **[ ]**
	SRC LogKow (KowWin) Program 1995
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	
Reference:	Meylan, W.M. and. P.H. Howard, 1995 J. Pharm. Sci. 84: 83-92

***2.6 WATER SOLUBILITY**

A. Solubility

Value:	15 ppm
Temperature:	25 °C
Description:	Miscible **[ ]**; Of very high solubility **[ ]**; Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble **[ ]**; Of low solubility **[ ]**; Of very low solubility **[X]**; Not soluble **[ ]**
Method:	Saturated Solution / Solvent Extraction / GC.Analysis
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	CH2Cl2 solvent, 100% recovery at 1 ppm. Equilibrated w/out light.
Reference:	Monsanto ES-78-SS-20, Environmental Sciences, 1978

B. pH Value, pKa Value

pH Value:	Not Applicable
pKa value	5.1 at 25°C
Method:	Estimated
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	Value indicates that this compound will exist only slightly in the cation form
Reference:	HSDB database 101-72-4, SRC, University of Georgia SPARC SPARC On-Line Calculator

2.11 OXIDISING PROPERTIES

Results:	Maximum burning rate equal or higher than reference mixture**[ ]**; Vigorous reaction in preliminary test **[ ]**; No oxidising properties **[ ]**; Other **[ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[ ]**
Remarks:	
Reference:	

†2.12 OXIDATION: REDUCTION POTENTIAL

Value:	. . . . . mV
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[ ]**
Remarks:	
Reference:	

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

Value:	
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[ ]**
Remarks:	
Reference:	

B. Other data

Results:	Henry's Law Constant = 1.4 x 10(-9) atm-cu m/mole

Remarks: Fragment Constant Estimation method. Volitazation from moist soil surfaces is not expected to be an important fate process.
Reference: HSDB – Lyman, W.J. et. al. Handbook of Chemical Property Estimation Methods, 1990

3. ENVIRONMENTAL FATE AND PATHWAYS

*3.1.1 PHOTODEGRADATION

Type: Air **[X]**; Water **[ ]**; Soil **[ ]**; Other **[ ]**
Light source: Sunlight **[ ]**; Xenon lamp **[ ]**; Other **[ ]**
Light spectrum: . . . . . nm
Relative intensity: . . . . . *(based on intensity of sunlight)*
Spectrum of substance: . . . . . nm
Concentration of Substance: . . . . . .
Temperature: . . . . . °C
Direct photolysis:
Half life: . . . . . .
Degradation: . . . . . % (weight/weight) after . . . . . (exposure time)
Quantum yield: . . . . . .
Indirect Photolysis:
Type of sensitizer: . . . . . .OH . . .
Concentration of sensitizer: . . 1560000 . . molecule/. cm^3 . . . . .
Rate constant (radical): . . . 218.3766 E-12. . . cm^3/molecule*sec
Degradation: . . . 50% at 0.588 Hrs . . . . . .
Method: calculated **[X]**; AOP Program (v1.89)
measured **[ ]**
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: . molecular structure. . . . . , purity:. . . . . .
Remarks:
Reliability: (2) valid with restrictions
Accepted calculation method
Reference: Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

*3.1.2 STABILITY IN WATER

Type: Abiotic (hydrolysis) **[X]**; biotic (sediment)**[ ]**
Half life: Not Determined
Degradation: 99% at pH 7.0 at 25 °C after 24 Hours
Method: Phase I Hydrolysis Study / ID of Hydrolysis Products
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex IP purple solid Lot # ND02-740, purity: >95%
Remarks: Rapid hydrolysis to benzoquinoneimine-N-phenyl and 4-hydroxy-diphenylamine. No starting material was detected by GC analysis after 7 days.
Reference: Monsanto ABC-32301, Analytical Bio-Chemistry Labs, 1986

*3.2 MONITORING DATA (ENVIRONMENTAL)

Type of Measurement: Background **[ ]**; At contaminated site **[ ]**; Other **[ ]**
Media:

Results:
Remarks:
Reference:

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

*3.3.1 TRANSPORT

Type: Adsorption **[ ]**; Desorption **[ ]**; Volatility **[ ]**; Other **[ ]**
Media:
Method:
Results:
Remarks:
Reference:

*3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota **[ ]**; Air-biota-sediment-soil-water **[ ]**; Soil-biota **[ ]**; Water-air **[ ]**; Water-biota **[ ]**; Water-soil **[ ]**; Other **[ ]**

Method: Fugacity level I **[ ]**; Fugacity level II **[ ]**; Fugacity level III **[X]**; Fugacity level IV **[ ]**; Other (calculation) **[ ]**; Other (measurement)**[ ]**

Results:

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	0.0158	1.18	1000	4.69e-013
Water	22.4	900	1000	1.97e-014
Soil	76.9	900	1000	3.94e-014
Sediment	0.68	3.6e+003	0	1.51e-014

	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	257	4.36	8.57	0.145
Water	1478	620	15.9	20.7
Soil	1.64e+003	0	54.6	0
Sediment	3.62	0.376	0.121	0.0125

Persistence Time: 922 hr
Reaction Time: 1.16e+003 hr
Advection Time: 4.42e+003 hr
Percent Reacted: 79.2
Percent Advected: 20.8

Remarks:
Reliability: (2) valid with restrictions
Accepted calculation method
Reference: Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

*3.5 BIODEGRADATION

Type: aerobic **[X]**; anaerobic **[ ]**
Inoculum: adapted **[X]**; non-adapted **[ ]**;
Concentration of the chemical: 1002 ug/l. related to COD **[]**; DOC **[]**; test substance**[X]**
Medium: water **[X];** water-sediment **[ ];** soil **[ ];** sewage treatment **[ ]**
Degradation: 50% after 2.5 Hours
90 % after 3.5 Hours

98% after 22 Hours

Results: readily biodeg. **[X];** inherently biodeg. **[ ]**; under test condition no biodegradation observed **[ ]**, other **[ ]**

Method: Natural Water Die-Away Test, Dixon, Hicks and Michael, 1981

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance: Santoflex IP purple solid Lot# N76-7433, purity:>95%.

Remarks: Tests run in Mississippi River Water and purified water. The short half-lives in both systems suggest that the compound should not persist in natural aquatic environments.

Reference: Monsanto ES-81-SS-53, MIC Environmental Sciences, 1981

4. ECOTOXICITY

*4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other*)* **[ ]**
open-system **[ ]**; closed-system **[X]**

Species: Salmo gairdneri (Rainbow Trout)

Exposure period: 96 Hours

Results: LC_{50} (24h) = 0.62 mg/l
LC_{50} (48h) = 0.38 mg/l
LC_{50} (72h) = Not reported
LC_{50} (96h) = 0.34 mg/l
NOEC = 0.18 mg/l
LOEC = 0.24 mg/l

Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**

Test substance: Santoflex IP dark solid, Lot#NO12-002, purity: >95%

Remarks: Stock solutions prepared in reagent-grade acetone. Water quality parameters of temperature, dissolved oxygen and pH monitored throughout test. Observations and mortality counts were made every 24 hours.

Reference: Monsanto BN-76-255, EG&G Bionomics, 1977

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other*)* **[ ]**
open-system **[ ]**; closed-system **[X]**

Species: Lepomis machrochirus (Bluegill Sunfish)

Exposure period: 96 Hours

Results: LC_{50} (24h) = 0.48 mg/l
LC_{50} (48h) = 0.43 mg/l
LC_{50} (72h) = Not reported
LC_{50} (96h) = 0.43 mg/l
NOEC = 0.24 mg/l
LOEC = 0.32 mg/l

Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**

Test substance: Santoflex IP dark solid, Lot# NO12-002, purity: >95%

Remarks: Stock solutions prepared in reagent-grade acetone. Water quality parameters of temperature, dissolved oxygen and pH monitored

throughout test. Observations and mortality counts were made every 24 hours

Reference: Monsanto BN-76-255, EG&G Bionomics, 1977

Type of test: static **[ ]**; semi-static **[ ]**; flow-through **[X]**; other **[ ]**
open-system **[ ]**; closed-system **[X]**
Species: Pimephales promelas (Fathead Minnows)
Exposure period: 14 days
Results: LC_{50} (24h) = 1.80 mg/l
LC_{50} (192h) = 0.28 mg/l
LC_{50} (240h) = 0.21 mg/l
LC_{50} (336h) = 0.09 mg/l
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex IP dark solid rec'd 4/25/78, purity: >95%
Remarks: Stock solutions prepared in reagent-grade acetone. Water quality parameters of temperature, dissolved oxygen, ammonia and pH monitored throughout test. Although the goal of the study was to determine a lethal threshold concentration of the test substance, the results indicated that this was not reached at 14 days. In addition, the test substance appeared to exhibit cumulative toxicity to the fish under test conditions.
Reference: Monsanto AB78-120B, Analytical Bio-Chemistry Labs, 1979

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other*)* **[ ]**
open-system **[ ]**; closed-system **[X]**
Species: Paratanytarsus parthenogenetica (Midge)
Exposure period: 48 Hours
Results: LC_{50} (24h) = 29 mg/l
LC_{50} (48h) = 23 mg/l
NOEC = Not Observed
LOEC = 10 mg/l (lowest concentration tested)
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975) and Gettings and Adams, Method for Conducting Acute Toxicity Tests with Midge 1980
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex IP #1803025-C), purity: >95%
Remarks: Stock solutions prepared in reagent-grade acetone. Water quality parameters of temperature, dissolved oxygen, ammonia and pH monitored throughout test.
Reference: Monsanto 9AB981013, Analytical Bio-Chemistry Labs, 1981

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]**;
open-system **[ ]**; closed-system **[X]**

Species: Daphnia magna
Exposure period: 48 Hours
Results: EC_{50} (24h) = 2.8 mg/l
EC_{50} (48h) = 1.1 mg/l
NOEC = 0.56 mg/l
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance: Santoflex IP purple flakes Lot #676-7433, purity: >95%
Remarks: Acetone used to prepare stock solutions. Initial range-finding experiment run to determine appropriate concentrations for final experiment. Water quality parameters of dissolved oxygen, pH, hardness, temperature and alkalinity monitored throughout the test.
Reference: Monsanto AB-78-120, Analytical Bio-Chemistry Labs, 1978

***4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae**

Species: Selanastrum capricurnutum (Freshwater alga)
Endpoint: Biomass **[ ]**; Growth rate **[X]**; Other **[ ]**
Exposure period: 96 Hours
Results: EC_{50} (96h) = 0.4 ppm for a chlorophyll, 0.5 ppm for cell numbers
NOEC = <0.1 ppm
LOEC = Not Determined
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: US EPA Algal Test Procedure: Bottle Test, 1971

open-system **[ ]**; closed-system **[X]**
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance: Santoflex IP #BN-78-1384325, purity: >95%
Remarks: Both a chlorophyll and cell numbers measured to confirm results. Stock solutions prepared in acetone; acetone also used as solvent control Concentrations of test article determined by preliminary range-finding experiment.
Reference: Monsanto BN-78-1384325, EG&G Bionomics, 1978

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Type: LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain: Sprague-Dawley Albino Rats
Value: 900 mg/kg b.w.:
Discriminating dose: 1000 mg/kg/bw
Method: Defined Lethal Dose
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance: Santoflex IP Lot# NO12-002, purity: <95%
Remarks: The test article was administered to groups of male and female rats by oral gavage as a 20% suspension in corn oil vehicle. Dose levels were 631, 794, 1000 or 1260 mg/kg/bw. Clinical signs of toxicity were reduced appetite and activity – three to five days in

survivors – followed by increasing weakness, collapse and death. Most deaths occurred within two days. Gross autopsy findings on decedents included lung hyperemia, slight liver discoloration and acute gastrointestinal inflammation. Survivors were sacrificed after a two-week recovery period. All viscera examined appeared normal in these animals.

Reference: Monsanto Y-73-287, Younger Laboratories, 1974

5.1.2 ACUTE INHALATION TOXICITY

Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[ ]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain:
Exposure time:
Value:
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance: . . . . . , purity: . . . . . .
Remarks:
Reference:

5.1.3 ACUTE DERMAL TOXICITY

Type: LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain: New Zealand Albino Rabbits
Value: >7940 mg/kg b.w.
Method: Defined Lethal Dose
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance: Santoflex IP Lot #NO12-002, purity: >95%
Remarks: The test article was applied to the shaved skin of groups of male and female rabbits for 24-hours as a 40% suspension in corn oil. Doses wer either 5010 or 7940 mg/kg/bw. All animals survived until sacrifice. Clinical signs of toxicity were limited to reduced appetite and activity for three to five days. Following a two-week recovery period, the animals were sacrificed. All viscera examined appeared normal in all animals.
Reference: Monsanto Y-73-287, Younger Laboratories, 1974

***5.4 REPEATED DOSE TOXICITY**

Species/strain: Sprague-Dawley Albino Rats
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral/Dietary
Exposure period: 30 Days
Frequency of treatment: Daily
Post exposure observation period:
Dose: 0, 500, 1000, 1750 or 2500 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[]**
NOEL: 500 ppm
LOEL: 1000 ppm
Results: In a 30-day range-finding study that preceeded a 90-day study, the test substance was administered orally, via dietary admixture, to groups of male and female rats (5/sex/group). Control animals received the standard laboratory diet. Physical observations, body weight and food consumption measurements were

performed on all animals pretest and at selected intervals during the study. Hematology and chemistry determinations were performed on all animals at study termination. There were no mortalities during the course of the study. After four weeks of treatment, all animals were sacrificed, selected organs were weighed, and organ/body weight ratios were calculated. Complete postmortem examinations were conducted on all animals. Differences from control in body weight gain, hematological effects, elevations in total serum protein and increased liver and spleen weights were noted in animals dosed at 1000 ppm and above. There were no significant differences in findings between control groups animals and those dosed at 500 ppm that were attributed to the test article.

Method: Dunnett, C.W., A Multiple Comparison Procedure for Comparing Several Treatments with a Control, Jour. Am. Stat. Assoc. 50: 1096-1121, 1955

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance: Santoflex IP Lot# 7J111, purity: 97.2%

Reference: Monsanto BD-88-74, Bio/dynamics Inc. 1988

Species/strain: Sprague-Dawley Albino Rats

Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**

Route of Administration: Oral/Dietary

Exposure period: 90 Days

Frequency of treatment: Daily

Post exposure observation period:

Dose: 0, 180, 360 or 720 ppm

Control group: Yes **[ ]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[]**

NOEL: 180 ppm for males, Not determined for females

LOEL: 360 ppm for males, 180 ppm for females

Results: The test substance was administered orally, via dietary admixture, to groups of male and female rats (10/sex/group). Control animals received the standard laboratory diet. Physical observations, body weight and food consumption measurements were performed on all animals pretest and at selected intervals during the study. Hematology and chemistry determinations were performed on all animals at Months 1.5 and 3. One high-dose and one mid-dose female were found dead on test day 93 following collection of terminal blood samples. The cause of death was attributed to the stress of bleeding and not to the administration of the test article. There were no other mortalities during the course of the study. After three months of treatment, all animals were sacrificed, selected organs were weighed, and organ/body and organ/brain weight ratios were calculated. Complete postmortem examinations were conducted on all animals. Histopathological evaluation of selected tissues was performed on all control and high-dose animals. The lungs, spleen, liver and kidneys were examined microscopically for all animals in all groups. Mean body weights and mean body weight gains were slightly reduced (2-4%) in males at 750 ppm.

Treatment-related findings were observed in several hematology parameters in the males and/or females at dose levels of 360 and 720 ppm. Parameters affected included reduced hemoglobin concentrations and hematocrit values at Week 6, reduced hemoglobin concentration in 720 ppm females at Week 13, elevated platelet counts in males at Week 6, and reduced mean erythrocyte counts in females at Week 6 and in high-dose females only at Week 13. The NOEL for hematology data was set at 180 ppm for both sexes. Differences in clinical chemistry parameters were noted in all mid- to high-dose animals. Mean liver weights, liver-to-body-weight and liver-to-brain-weight ratios were increased in 360 and 720 males, and in all treated females. There were no treatment-related findings noted in mortality, physical observations, opthalmology, food consumption or gross or microscopic pathology in any dose/sex group.

Method: OECD Guidelines for Testing of Chemicals, Section 453, 1981 and US EPA TSCA Section 4(a) Test Rules, 1982

GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance: Santoflex IP Lot# 7J111, purity: 97.2%

Reference: Monsanto BD-88-389, Bio/dynamics, Inc. 1990

*5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

Type: Bacterial Reverse Mutation - Ames

System of testing: TA-98, TA-100, TA-1535, TA-1537, TA-1538

Concentration: 0.1, 1,10, 100 and 500 micrograms/plate

Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**

Results:

Cytotoxicity conc: With metabolic activation: 500 ug/plate
Without metabolic activation: 500 ug/plate

Precipitation conc: Not determined

Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[X]**
Without metabolic activation:	**[ ]**	**[ ]**	**[X]**

Method: Ames Plate Test (Overlay method) 1975; OECD 471 equivalent

GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**

Test substance: Santoflex IP 11-02-76, purity: >95%

Remarks: Stock solutions prepared in DMSO. No evidence of mutagenic activity in any assay conducted with or without activation using the S-9 homogenate from Arochlor-induced rat livers.

Reference: Monsanto BIO-76-226, Litton Bionetics, 1976

Type: Bacterial Reverse Mutation - Ames

System of testing: TA-98, TA-100, TA-1535, TA-1537

Concentration: 0.2, 0.8, 4, 20, 60 and 200 micrograms/plate

Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**

Results:

Cytotoxicity conc: With metabolic activation: 200 ug/plate

Without metabolic activation: 200 ug/plate
Precipitation conc: Insoluble at 1 mg/plate and above
Genotoxic effects: + ? -
With metabolic activation: **[ ] [ ] [X]**
Without metabolic activation: **[ ] [ ] [X]**
Method: Ames Plate Test (Overlay method) 1975; OECD 471 equivalent
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex IP Lot# ND02-740, purity: 92-99%
Remarks: Stock solutions prepared in DMSO. No evidence of mutagenic activity in any assay conducted with or without activation using the S-9 homogenate from Arochlor-induced rat livers.
Reference: Monsanto ML-85-243, Environmental Health Labs, 1986

B. NON-BACTERIAL IN VITRO TEST

Type: Mammalian Cell Gene Forward Mutation Assay
System of testing: L5178Y Mouse Lymphoma cells
Concentration: 0.156, 0.313, 0.625, 1.250, 2.500 (without activation)
0.625, 1.250, 2.500, 5.000 and 10.000 (with activation)
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:
Cytotoxicity conc: With metabolic activation: 10.0 ug/ml
Without metabolic activation: 2.5 ug/ml
Precipitation conc: >1 mg/ml
Genotoxic effects: + ? -
With metabolic activation: **[ ] [ ] [X]**
Without metabolic activation: **[ ] [ ] [X]**
Method: Clive and Spector, Mutation Research 31:17-29 (1975)
GLP: Yes **[ ]** No **[ ]** ? **[X]** **Klimisch 2**
Test substance: Santoflex IP flakes Lot # N76-7433, purity 97%
Remarks: The test article was evaluated for specific locus forward mutation in the L5178Y Thymidine Kinase (TK) mouse lymphoma cell assay. Stock solutions were prepared in DMSO. DMSO was used as the negative control. EMS was used as the positive control without activation and DMN was used as the positive control with activation. The test article was found to be negative
Reference: Monsanto BIO-78-224 Litton Bionetics, 1978

Type: *In vitro* Unscheduled DNA Synthesis (UDS)
System of testing: Primary rat hepatocyte cultures (Fischer-344 strain)
Concentration: 0.01, 0.05, 0.1, 0.5, 1, 3, 5, 10, 50, 100, 1000 ug/ml
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:
Cytotoxicity conc: Preliminary Assay: 5 ug/ml
Replicate Assay: 3 ug/ml
Precipitation conc: Separation/sticking to sides of tube noted at 100 ug/ml and above
Genotoxic effects: + ? -
[ ] [ ] [X]
Method: Williams, G.M., Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures, Cancer Research 37, pp. 1845-1851 (1977)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**

Test substance: Santoflex IP flakes Lot# ND02-740, purity 92-97%
Remarks: Acetone (1%) used as solvent and diluent. Primary rat liver cell cultures derived from the livers of two adult male rats. The positive control was 2-AAF, the solvent control was acetone in the preliminary assay and DMSO in the replicate assay. The percentage of cells in repair was calculated as the percentage of cells with at least 5 net grains/nucleus. 150 cells were scored for each concentration reported for each experiment. The net grain counts were negative at each concentration of the test compound, in the solvent control and in the medium control, in contrast to the strong positive response produced by the positive control 2-AAF in both experiments. These results indicate that Santoflex IP is not a genotoxic agent under the conditions of the in vitro rat hepatocyte DNA repair assay.
Reference: Monsanto SR-85-251, SRI International, 1986

Type: CHO/HGPRT Forward Gene Mutation Assay
System of testing: Cultured Chinese hamster ovary (CHO) cells
Concentration: 2, 5, 10, 15 and 30 ug/ml
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:
Cytotoxicity conc: With metabolic activation: 30 ug/ml
Without metabolic activation: 10 ug/ml
Precipitation conc: Not Determined
Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[X]**
Without metabolic activation:	**[ ]**	**[ ]**	**[X]**

Method: CHO/HGPRT Mutation Assay (1979) Hsie, et.al.
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex IP Lot# N002-740, purity: 92-99%
Remarks: The mutagenic potential of Santoflex IP was tested in CHO cells for ability to induce forward mutation at the HGPRT gene locus. A range-finding cytotoxicity study preceded a dose-response mutagenicity experiment using different levels of Arochlor1254 rat liver homogenate (S9) concentrations, followed by a confirmatory dose-response mutagenicity experiment. The compound was tested at S9 concentrations up to a cytotoxic dose of 30 ug/ml. No statistically significant mutagenicity was observed in the two separate experiments. Therefore, the test substance was not considered to be mutagenic in CHO cells under the experimental conditions.
Reference: Monsanto ML-85-221, Environmental Health Labs, 1986

*** 5.6 GENETIC TOXICITY IN VIVO**

Type:
Species/strain:
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration:
Exposure period:
Doses:
Results:

Effect on mitotic
index or P/N ratio:
Genotoxic effects: + ? -
[ ] [ ] [ ]

Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance: . . . . . , purity: . . . . . .
Remarks:
Reference:

***5.8 TOXICITY TO REPRODUCTION**

Type: Fertility **[]**; One-generation study **[]**; Two-generation study **[]**; Other **[ ]**
Species/strain:
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration:
Exposure period:
Frequency of treatment:
Post exposure observation period:
Premating exposure period: male: . . . . . , female:
Duration of the test: .
Doses:
Control group: Yes **[ ]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Parental:
NOEL F1 Offspring:
NOEL F2 Offspring:
Results: General parental toxicity
Toxicity to offspring:
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance: . . . . . , purity: . . . . . .
Remarks:
Reference:

***5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY**

Species/strain: Sprague-Dawley CD Rats
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Oral gavage
Duration of the test: 20 days from mating to C-section
Exposure period: Day 6-15 of gestation
Frequency of treatment: Daily, as a single oral dose at a volume of 5 ml/kg
Doses: 0, 12.5, 62.5 and 125 mg/kg/bw
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[]**; Concurrent vehicle **[X]**; Historical **[]**
NOEL Maternal Toxicity: 62.5 mg/kg
NOEL teratogenicity : 62.5 mg/kg
Results: The test substance was administered to groups of 24 pregnant rats during the period of embryo organogenesis. The vehicle was Polyethylene Glycol 400, and dose levels were 0, 12.5, 62.5 or 125 mg/kg/bw.

Maternal general toxicity: High-dose rats exhibited slight maternal toxicity as evidenced by a reduction in food intake, pre-dosing salivation and soft, dark feces. There were no effects on body weight. All animals survived to sacrifice. There were no treatment-related macroscopic findings at necropsy for any dose level.
Pregnancy/litter data: There were no treatment-related effects on uterine/implantation.
Foetal data: At 125 mg/kg there were statistically significant effects on the incidence of skeletal findings. Effects included an increased incidence of irregularly and incompletely ossified cranial and facial bones, and increased incidence of no ossification of hyoid, unilateral/bilateral wavy ribs, and semi-bipartite vertebral centra. At 62.5 mg/kg, there was a statistically significant increase in incomplete ossification of more than one cranial bone. At 12.5 mg/kg, there was a statistically significant increase in the incomplete ossification of more than one facial bone that was not considered to be treatment-related.

Method: OECD 59B (1982)
GLP: Yes **[X]** No **[ ]** ? **[ ]** **Klimisch 1**
Test substance: Santoflex IP dark flakes, Lot#2F054, purity: 97%
Remarks: No deviations from protocol noted.
Reference: Monsanto SP-93-46, SafePharm Laboratories 1994

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

Type: Immunotoxicity
Repeat Insult Patch Test

Results: Santoflex IP, 50% w/v in Dimethylphthalate, was applied to the upper arm of 50 human volunteers using a linteen disk moistened with the test material. The patch was kept in place for 24 hours before removal and grading of gross skin changes on a scale of 0-4. After a 24-hour rest period, the test material was reapplied. This cycle was repeated every Monday, Wednesday and Friday, with a 48-hour rest period over weekends. After the 15th application, the volunteers rested two weeks before the challenge application.

Application #1:	Score 0/50
Applications #2-15:	Score 10/50
Challenge:	Score 11/50

Remarks: Under the test conditions, 11/50 or 22% of the volunteers showed sensitization responses. Those 11 persons were also subjected to a supplementary challenge using Santoflex 13 (6PPD). No subject showed any indication of cross-sensitization from one PPD rubber chemical material to another.
Reference: Monsanto SH-76-7, Product Investigations, Inc. 1976

Type: Immunotoxicity
Modified Draize Skin Sensitization Study on Human Volunteers
Results: The study was performed over a 6-week period on 82 human

volunteers using Santoflex IP, 1%, in petrolatum. During the first three weeks, patches moistened with the test material were applied to the arms at the same site at the rate of three times/week. Following a rest period, a challenge application was made to a different site. Results for irritation and sensitization were scored on a scale of 0-4. 12 of 82 test subjects were deemed to be sensitized, for a rate of 14.6%

Reference: Monsanto MA-78-92, 1978

B. Toxicodynamics, toxicokinetics

Type: .
Results:
Remarks:
References:

*** 5.11 EXPERIENCE WITH HUMAN EXPOSURE**

Results:
Remarks:
Reference:

6. REFERENCES

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2. Monsanto Toxicology Profile, Physical Properties of Santoflex IP Antiozonant, R.M. Bannister, January 2, 1990
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7. University of Georgia SPARC On-Line Calculator, June 2000
8. Lyman, W.J. et. al., Handbook of Chemical Property Estimation Methods, Washington, D.C., American Chemical Society, pp 4-9 15-1 to 15-29, 1990
9. Monsanto ABC-32301, Santoflex IP Phase I Hydrolysis Study: Identification of Hydrolysis Products, Analytical Bio-Chemistry Laboratories, Inc., March 10, 1986
10. Monsanto ES-81-SS-53, Environmental Fate Screening of Santoflex IP, Monsanto Industrial Chemical Environmental Sciences Laboratories, December 31, 1981
11. Monsanto BN-76-255, Acute (96 hour) Toxicity of Santoflex IP to Rainbow Trout and Bluegill, EG&G Bionomics Aquatic Toxicity Laboratory, January 1977
12. Monsanto AB-78-120B, Dynamic Toxicity of Santoflex IP to Fathead Minnows, Analytical Bio-Chemistry Laboratories, Inc. July 30, 1979
13. Monsanto 9AB981013, Acute Toxicity of Santoflex IP to Midge, Analytical Bio-Chemistry laboratories, Inc. September 18, 1981
14. Monsanto AB-78-120, Acute Toxicity of Santoflex IP to Daphnia magna, Analytical Bio-Chemistry Laboratories, Inc. August 25, 1978
15. Monsanto BN-78-1384325, Acute Toxicity of Santoflex IP to the Freshwater Alga Selenastrum capricornutum, EG&G Bionomics Marine Research Laboratory, September 1978
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18. Monsanto BD-88-74, A One-Month (30 Days) Oral Toxicity Study with Santoflex IP in the Rat via Dietary Admixture, Bio/dynamics Inc, August 1988
19. Monsanto BD-88-389, A Subchronic (3 Month) Oral Toxicity Study with Santoflex IP in the Rat via Dietary Admixture, Bio/dynamics, Inc., June 1990
20. Monsanto BIO-76-226, Mutagenicity Evaluation of Santoflex IP, Litton Bionetics, Inc. December 30, 1976
21. Monsanto ML-85-243, Ames/Salmonella Mutagenicity Assay of Santoflex IP, Monsanto Environmental Health Laboratory, February 18, 1986
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25. Monsanto SP-93-46, IPPD: Oral Gavage Teratology Study in the Rat, SafePharm Laboratories, January 27, 1994
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I U C L I D

D a t a S e t

Existing Chemical	ID: 793-24-8
CAS No.	793-24-8
EINECS Name	N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine
EINECS No.	212-344-0
TSCA Name	1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl-
Molecular Formula	C18H24N2

Producer Related Part	
Company:	
Creation date:	23-SEP-1999
Substance Related Part	
Company:	
Creation date:	23-SEP-1999
Memo:	RAPA PPD category
Printing date:	20-NOV-2001
Revision date:	
Date of last Update:	20-NOV-2001
Number of Pages:	57
Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 OECD and Company Information

Type: lead organisation
Name: American Chemistry Council (formerly Chemical Manufacturers Association), Rubber and Plastic Additives Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States
Phone: 703-741-5600
Telefax: 703-741-6091

20-NOV-2001

Type: cooperating company
Name: Bayer Corporation
Country: United States

20-NOV-2001

Type: cooperating company
Name: Ciba Specialty Chemicals Corporation
Country: United States

20-NOV-2001

Type: cooperating company
Name: Crompton Corporation
Country: United States

20-NOV-2001

Type: cooperating company
Name: Flexsys America L.P.
Country: United States

20-NOV-2001

Type: cooperating company
Name: Noveon, Inc. (formerly BF Goodrich)
Country: United States

20-NOV-2001

Type: cooperating company
Name: R.T. Vanderbilt Company, Inc.
Country: United States

20-NOV-2001

Type: cooperating company
Name: The Goodyear Tire & Rubber Company
Country: United States

20-NOV-2001

Type: cooperating company
Name: The Lubrizol Corporation
Country: United States

20-NOV-2001

Type: cooperating company
Name: UOP, LLC.
Country: United States

20-NOV-2001

1.0.2 Location of Production Site
-

1.0.3 Identity of Recipients
-

1.1 General Substance Information
-

1.1.0 Details on Template
-

1.1.1 Spectra
-

1.2 Synonyms
-

1.3 Impurities
-

1.4 Additives
-

1.5 Quantity
-

1.6.1 Labelling
-

1.6.2 Classification
-

1.7 Use Pattern
-

1.7.1 Technology Production/Use
-

1.8 Occupational Exposure Limit Values
-

1.9 Source of Exposure
-

1.10.1 Recommendations/Precautionary Measures
-

1.10.2 Emergency Measures
-

1.11 Packaging
-

1.12 Possib. of Rendering Subst. Harmless
-

1.13 Statements Concerning Waste
-

1.14.1 Water Pollution
-

1.14.2 Major Accident Hazards
-

1.14.3 Air Pollution
-

1.15 Additional Remarks
-

1.16 Last Literature Search
-

1.17 Reviews
-

1.18 Listings e.g. Chemical Inventories
-

2.1 Melting Point

Value: 45 degree C
Decomposition: no
Sublimation: no
Method: other: FF83.9-1 Initial and Final Melting Point of Organic Compounds.
Year: 1996
GLP: yes
Testsubstance: other TS: CAS# 793-24-8
Remark: Capillary method
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
20-NOV-2001 (1)

Value: 50 degree C
Method: other: Handbook value
GLP: no data
Testsubstance: other TS: CAS# 793-24-8
Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
20-NOV-2001 (2)

Value: 45 - 48 degree C
Source: Bayer AG Leverkusen
20-NOV-2001 (3)

2.2 Boiling Point

Value: 230 degree C at 13.3 hPa
Source: Bayer AG Leverkusen
28-SEP-1992 (3)

2.3 Density

Type: relative density
Value: 1 at 15 degree C
Method: other: FF97.8-1 Flexsys Standard Method
Year: 1997
GLP: yes
Testsubstance: other TS: CAS# 793-24-8
Remark: Density of solids by displacement
Flag: Critical study for SIDS endpoint
20-NOV-2001 (4)

Type:
Value: 1.02 g/cm3 at 20 degree C
Source: Bayer AG Leverkusen
28-SEP-1992 (3)

Type:
Value: .995 g/cm3 at 50 degree C
Source: Bayer AG Leverkusen
20-JUN-1997 (5)

Type: relative density
Value: 1 at 60 degree C
Source: MonsantoBayer AG Leverkusen
26-MAY-1994

2.3.1 Granulometry
-

2.4 Vapour Pressure

Value: 8.7 hPa at 200 degree C
Source: Bayer AG Leverkusen
28-SEP-1992 (3)

Value: 93 hPa at 300 degree C
Source: Bayer AG Leverkusen
28-SEP-1992 (3)

2.5 Partition Coefficient

log Pow: 4.68 at 25 degree C
Method: other (calculated): SRC LogKow (KowWin) Program
Year: 1995
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
20-NOV-2001 (6)

log Pow: 5.4
Method: other (calculated): Leo, A.: CLOGP-3.54 MedChem Software 1989. Daylight, Chemical Information Systems, Claremont, CA 91711, USA
Year:
Source: Bayer AG Leverkusen
Reliability: (2) valid with restrictions
20-NOV-2001 (7)

log Pow:
Method:
Year:
Remark: pow = 59000 +/- 34000
Source: Bayer AG Leverkusen
14-JAN-1993 (8)

2.6.1 Water Solubility

Value:	1.1 other: ppm at 23 degree C
Qualitative:	not soluble
Method:	other: Saturated Solution / Solvent Extraction / GC.Analysis
GLP:	no data
Testsubstance:	other TS: CAS# 793-24-8
Remark:	CH2Cl2 solvent, 96% recovery at 1 ppm. Equilibrated w/out light.
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag:	Critical study for SIDS endpoint

20-NOV-2001 (9) (10)

Value:	ca. 1 mg/l at 50 degree C
Method:	other: modified OECD Guideline 105 "Water solubility-Flask Method"
Source:	Bayer AG Leverkusen
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment

20-NOV-2001 (5)

2.6.2 Surface Tension

-

2.7 Flash Point

Value:	200 degree C
Type:	closed cup
Method:	other: DIN 51758
Year:	
Source:	Bayer AG Leverkusen

28-SEP-1992 (3)

2.8 Auto Flammability

-

2.9 Flammability

Result:	
Remark:	no information
Source:	Bayer AG Leverkusen

04-FEB-1992

2.10 Explosive Properties

-

2.11 Oxidizing Properties
-

2.12 Additional Remarks
-

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000 molecule/cm3
Rate constant: .0000000002264928 cm3/(molecule * sec)
Degradation: 50 % after .6 hour(s)
Method: other (calculated): AOP Program (v1.89)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
20-NOV-2001 (11)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (calculated): calculation according to Atkinson
Year: GLP:
Test substance: other TS: CAS# 793-24-8
Remark: t1/2 = 1.1 h
Source: Bayer AG Leverkusen
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
20-NOV-2001

3.1.2 Stability in Water

Type: abiotic
Degradation: 93 % after 24 hour(s)
at pH 70 and 25 degree C
Deg. Product: yes
Method: other: Phase I Hydrolysis Study / ID of Hydrolysis Products
Year: GLP: yes
Test substance: other TS: Purple solid # KD08-281 purity: >95%
Remark: Rapid hydrolysis to 4-Hydroxylamine and
Benzoquinoneimine-N-phenyl.
Reliability: (1) valid without restriction
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
20-NOV-2001 (12)

Type: abiotic
Degradation: = 60 % after 25 hour(s)
Method: other: Monsanto Laboratory protocol; see test conditions
Year: 1978 GLP: no data
Test substance:
Remark: Degradation data versus time: 0 hour 1 mg/l, 1 hour 0.855 mg/l, 2 hour 0.846 mg/l, 3.5 hour 0.636 mg/l and 25 hour 0.402 mg/l
Source: MonsantoBayer AG Leverkusen
Test condition: Degradation of test substance in deionized water
Reliability: (2) valid with restrictions
20-OCT-1999 (13)

Type: abiotic
t1/2 pH7: = 3 - 4 hour(s) at 24 degree C
Method: other: Monsanto Laboratory protocol; see test conditions
Year: 1993 GLP: yes
Test substance:
Remark: Santoflex 13 is an antiozonant and as such necessarily reacts very quickly with oxygen. Therefore, fast oxidation in dilute solutions, where oxygen is readily available, is to be expected. The initial oxidation product is believed to be quinondiimine, which itself is a very reactive species. The quinondiimine can hydrolyze or form a polymer by further oxidation giving very complicated mixtures of products usually involving loss of the alkyl group.
Source: MonsantoBayer AG Leverkusen
Test condition: Degradation in pH 7 buffered deionized water
30-MAY-1994 (14)

3.1.3 Stability in Soil

Type: Radiolabel:
Concentration:
Cation exch. capac.
Microbial biomass:
Method:
Year: GLP:
Test substance:
Remark: no information
Source: Bayer AG Leverkusen
12-JUN-1992

3.2 Monitoring Data (Environment)

Type of measurement:
Medium:
Method:
Concentration
Remark: no information
Source: Bayer AG Leverkusen
06-FEB-1992

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
Media: other: air, water, soil, sediment
Air (Level I):
Water (Level I):
Soil (Level I):
Biota (L.II/III):
Soil (L.II/III):
Method: other: EPIWIN Level III Fugacity Model
Year: 1999
Result:

Media	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	0.0264	1.13	1000	6.66e-013
Water	19.6	900	1000	3.36e-014
Soil	68.1	900	1000	2.84e-015
Sediment	12.2	3.6e+003	0	2.28e-014

Media	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	457	7.47	15.2	0.249
Water	427	555	14.2	18.5
Soil	1.48e+003	0	49.4	0
Sediment	66.2	6.88	2.21	0.229

Persistence Time: 941 hr
Reaction Time: 1.16e+003 hr
Advection Time: 4.96e+003 hr
Percent Reacted: 81
Percent Advected: 19

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
20-NOV-2001 (11)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water
Method: other (calculation): Fugacity Level III
Year: 1999
Result:

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.0264	1.13	1000
Water	19.6	900	1000
Soil	68.1	900	1000
Sediment	12.2	3600	0

Pers
Reliability: (2) valid with restrictions
21-OCT-1999 (15)

Media:
Method:
Year:
Remark: Based on the calculated log Pow, transport of the compound from water to soil/sediment (geoaccumulation) is to be expected.
Water solubility and vapour pressure indicate that the transport from water to air is of low relevance.
Source: Bayer AG Leverkusen
21-OCT-1999

3.4 Mode of Degradation in Actual Use

Remark: no information
Source: Bayer AG Leverkusen
06-FEB-1992

3.5 Biodegradation

Type: aerobic
Inoculum: other: Mississippi River water
Concentration: 1.002 mg/l related to Test substance
Degradation: = 97 % after 22 hour(s)
Result: other: Primary degradation, 96 % primary degradation in sterile river water and 88 % in deionized water in 22 hours
Testsubstance: 1 hour(s) = 40 %
2 hour(s) = 57 %
3 hour(s) = 67 %
4 hour(s) = 62 %
5 hour(s) = 74 %
Method: other: Natural Water Die-Away in Mississippi River water
Year: GLP: yes
Test substance: other TS: Santoflex 13 Lot# KD-03017, purity: >95%
Remark: Rate of disappearance in

time	active Mississippi River water	sterile Mississippi River water	deionized water
0 hour	100 %	100 %	100 %
1 hour	60 %	85 %	100 %
2 hour	43 %	70 %	88 %
3 hour	33 %	56 %	86 %
4 hour	38 %	49 %	80 %
5 hour	26 %	41 %	65 %
22 hour	3 %	4 %	12 %

Result: 50% degradation after 2.9 hours
Reliability: (1) valid without restriction
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
20-NOV-2001 (16)

Type: aerobic
Inoculum: predominantly domestic sewage
Degradation: 13 - 40 % after 28 day
Method: other: Respirometer-Test, ISO DP 9408, EG Directive 79/831/ Annex V, modified MITI Test
Year: GLP: no
Test substance: other TS
Source: Bayer AG Leverkusen
Test substance: technical grade 6PPD
20-NOV-2001 (17)

Type: aerobic
Inoculum: activated sludge
Concentration: 30 mg/l related to Test substance
Degradation: = 7.2 % after 32 day
Result: other: 7.2 % CO2 evolution in 32 days
Method: other: Method similar to Gledhill method listed in U.S.E.P.A. 40 CFR Ch 1 subpart D paragraph 796.3100.
Year: GLP: no data
Test substance:
Source: MonsantoBayer AG Leverkusen
20-NOV-2001 (13)

3.6 BOD5, COD or BOD5/COD Ratio
-

3.7 Bioaccumulation
-

3.8 Additional Remarks

Remark: 1.4-Benzenediamine, N-(1.3-dimethylbutyl)-N'-phenyl decreases the degradation rate of unprotected rubber (vulcanisate) in water.
Source: Bayer AG Leverkusen
01-DEC-1992 (18)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: = .14
Method: other: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians.
Year: 1977 GLP: no data
Test substance: other TS: Santoflex 13 Lot# KD03-017, purity: >95%.
Remark: Solutions in reagent-grade acetone; Water quality parameters monitored throughout test.
Result: 96 hr C.I. = 0.12 - 0.16 mg/l;
24 hr LC50 = 0.28 mg/l;
48 hr LC50 = 0.18 mg/l
Test condition: carrier-acetone; 15L water; 10 fish/vessel; length = 3.7 cm; no food; no aeration; temp = 12C
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
20-NOV-2001 (19)

Type: static
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: .4
Method: other: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians.
Year: 1977 GLP: no data
Test substance: other TS: Santoflex 13 Lot# KD03-017, purity: >95%.
Remark: Solutions in reagent-grade acetone; Water quality parameters monitored throughout test.
Result: 96 hr C.I. = 0.32 - 0.5 mg/l;
24 hr LC50 = 0.65 mg/l;
48 hr LC50 = 0.45 mg/l
Test condition: carrier-acetone; 15L water; 10 fish/vessel; length = 3.8 cm; no food; no aeration; temp = 22C
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
20-NOV-2001 (19)

Type: static
Species: Brachydanio rerio (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: 5
LC100: 100
Method: other: see remarks
Year: 1984 GLP: no
Test substance: other TS: technical grade 6PPD
Remark: following OECD 203
The powdered test substance was dispersed in water. LC-values given above are nominal concentrations: weight of the dispersed substance per liter water.
Source: Bayer AG Leverkusen
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
20-NOV-2001 (20)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 28 day
Unit: mg/l Analytical monitoring: yes
LC50: = .15
Method: other: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians.
Year: 1984 GLP: yes
Test substance: other TS: Santoflex 13 purity: >95%.
Remark: C.I. = 0.13 - 0.17 mg/l; 48 hr LC50 = 2 mg/l; 6, 7 and 8 day LC50 = 0.35 mg/l; 19, 20, 21 day LC50 = 0.17 mg/l
Tests in well water; Stock solutions in acetone; Water quality parameters monitored throughout test.
Result: 28D C.I. = 0.13 - 0.17 mg/l;
48 hr LC50 = 2 mg/l;
6, 7 and 8 day LC50 = 0.35 mg/l;
19, 20, 21 day LC50 = 0.17 mg/l
Reliability: (1) valid without restriction
GLP guideline study
20-NOV-2001 (21)

4.2 Acute Toxicity to Aquatic Invertebrates

Type:	static
Species:	Daphnia magna (Crustacea)
Exposure period:	48 hour(s)
Unit:	mg/l Analytical monitoring: yes
NOEC:	= .56
EC50:	= .82
Method:	other: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians
Year:	GLP: yes
Test substance:	other TS: Santoflex 13, purity: >95%
Remark:	Solutions in reagent-grade acetone; Water quality parameters monitored throughout test.
Result:	C.I. for 48 hr EC50=0.71-0.94 mg/l; 24 hr EC50=1 mg/l
Reliability:	(1) valid without restriction GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag:	Critical study for SIDS endpoint

20-NOV-2001 (22)

Type:	
Species:	Daphnia magna (Crustacea)
Exposure period:	48 hour(s)
Unit:	mg/l Analytical monitoring: no
NOEC:	= .4
EC50:	= .79
Method:	OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year:	1984 GLP: no data
Test substance:	
Remark:	C.I. for EC50 = 0.7 - 0.91 mg/l; 24 hr EC50=1.6 mg/l; 48 hr EC50=0.79 mg/l; in presence of food 48 hr EC50= 1.3 mg/l and NOEC=0.4 mg/l
Source:	MonsantoBayer AG Leverkusen
Test condition:	carrier-acetone; no food
Reliability:	(1) valid without restriction Guideline study
Flag:	Critical study for SIDS endpoint

20-NOV-2001 (23)

Type:
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: = .25
EC50: = .51
Method: OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year: 1984 GLP: no data
Test substance:
Remark: the test solution was allowed to age 40 hours before test 48 hr EC50>1 mg/l and NOEC>1 mg/l
Source: MonsantoBayer AG Leverkusen
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
20-NOV-2001 (24)

Type:
Species: other: Chironomus tentans
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: = .6
EC50: = .99
Method: other: EPA. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. EPA-660/3-75-009.
Year: 1975 GLP: no data
Test substance:
Remark: C.I. for EC50=0.6-1.25 mg/l; 24hr EC50=1.25 mg/l
Source: MonsantoBayer AG Leverkusen
Test condition: water solubility was exceeded at three highest concentrations; larvae 10-14 days old; room temp
30-MAY-1994 (25)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Selenastrum capricornutum (Algae)
Endpoint: biomass
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
EC50: = .6
Method: other: EPA Selenastrum capricornutum Algal Assay Test
Year: 1971 GLP: no data
Test substance: other TS: Santoflex 13 (Monsanto) purity: >95%
Remark: Phytotoxicity maxed at 48 hours; test solutions in acetone
Result: 96 hr C.I. 0.2-2 mg/l;
in vivo chlorophyll results-
24hr EC50=2.0 mg/l,
48hr EC50=0.5 mg/l,
72hr EC50=0.5 mg/l,
96hr EC50=0.6 mg/l
Test condition: temp=24C; 4000 lux; Algal Assay media; "cool" white lights;
init. inoc.=10000 cells/ml
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
20-NOV-2001 (26) (27)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type:
Species: activated sludge
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 420
Method: ISO 8192 "Test for inhibition of oxygen consumption by
activated sludge"
Year: GLP: no
Test substance: other TS
Source: Bayer AG Leverkusen
Test substance: technical grade 6PPD
01-DEC-1992 (20)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: no information
Source: Bayer AG Leverkusen
06-FEB-1992

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: no information
Source: Bayer AG Leverkusen
06-FEB-1992

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

Type:
Species:
Endpoint:
Exposure period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: no information
Source: Bayer AG Leverkusen
06-FEB-1992

4.6.2 Toxicity to Terrestrial Plants

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: no information
Source: Bayer AG Leverkusen
06-FEB-1992

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species
-

4.7 Biological Effects Monitoring

Remark: no information
Source: Bayer AG Leverkusen
06-FEB-1992

4.8 Biotransformation and Kinetics

Type:
Remark: no information
Source: Bayer AG Leverkusen
06-FEB-1992

4.9 Additional Remarks
-

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type:	LD50
Species:	rat
Strain:	Sprague-Dawley
Sex:	male/female
Number of Animals:	10
Vehicle:	
Value:	> 5000 mg/kg bw
Method:	other:EPA/TSCA Acute Oral Toxicity and the EEC Methods for Determining Toxicity, Part B.1, No. L 251/96 Sept. 1984
Year:	GLP: yes
Test substance:	other TS: 6PPD Ref# 4065459 solid, purity: 97.6%
Remark:	Following a range-finding study, 6PPD was fed to a group of five male and five female rats in a single oral dose of 5000 mg/kg body weight. Rats were observed daily and weighed weekly. 2 males and I female died prior to sacrifice. A gross necropsy examination was performed on all surviving animals at sacrifice on Day 15. Clinical findings included decreased fecal output, fecal/urine stains, rough coat, piloerection and soft stools. One male and three females showed weight loss; all other animals gained weight. Most notable internal necropsy finding was black, hard material in the stomach contents. Findings in animals that died included discolored mucoid contents throughout the digestive system with reddened mucosa/dark red foci of the stomach.
Reliability:	(1) valid without restriction GLP guideline study
Flag:	Critical study for SIDS endpoint

20-NOV-2001 (28)

Type:	LD50
Species:	rat
Strain:	
Sex:	
Number of Animals:	
Vehicle:	
Value:	= 3340 mg/kg bw
Method:	
Year:	GLP:
Test substance:	other TS: undiluted
Source:	Bayer AG Leverkusen

08-DEC-1992 (29)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 2500 mg/kg bw
Method:
Year: GLP:
Test substance:
Source: Bayer AG Leverkusen
08-DEC-1992 (30)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 3580 mg/kg bw
Method:
Year: GLP:
Test substance: other TS: purity 95.7 %
Source: Bayer AG Leverkusen
08-DEC-1992 (31)

Type: LD50
Species: mouse
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 3200 mg/kg bw
Method:
Year: GLP:
Test substance:
Source: Bayer AG Leverkusen
08-DEC-1992 (30)

Type: LD50
Species:
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 1120 mg/kg bw
Method:
Year: GLP:
Test substance:
Source: Bayer AG Leverkusen
08-DEC-1992 (32)

5.1.2 Acute Inhalation Toxicity
-

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: New Zealand white
Sex: male/female
Number of
Animals:
Vehicle: other: undiluted
Value: > 7940 mg/kg bw
Method: other: Defined Lethal Dose
Year: GLP: no data
Test substance: other TS: CP 22423 Lot# KC07-298, purity: >95%.
Remark: The undiluted test article was applied to the shaved skin of male and female rabbits at dose levels ranging from 3160 to 7940 mg/kg/bw. Clinical signs were reduced appetite and activity for three to seven days. All animals survived. Autopsy results showed that all viscera appeared normal.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
20-NOV-2001 (31)

Type: LDLo
Species: rabbit
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: 3160 - 5010 mg/kg bw
Method:
Year: GLP:
Test substance: other TS: undiluted
Source: Bayer AG Leverkusen

08-DEC-1992 (29)

5.1.4 Acute Toxicity, other Routes
-

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: slightly irritating
EC classificat.:
Method: Draize Test
Year: GLP:
Test substance: other TS: undiluted
Remark: method: the data were scored according to the method of Draize et al. (1944), 24 h exposure, then skin rinsed with warm water and soap, observation period 5 days
Source: Bayer AG Leverkusen
08-DEC-1992 (29)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: slightly irritating
EC classificat.:
Method: Draize Test
Year: GLP:
Test substance: other TS: 12.5 and 125 mg 6PPD dispersed in 0.5 g vaseline (2.5 and 25 %)
Remark: method: after 24 h and 72 h examination
Source: Bayer AG Leverkusen
08-DEC-1992 (33)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: moderately irritating
EC classificat.:
Method: Draize Test
Year: GLP:
Test substance: other TS: 25 mg 6PPD dispersed in 0.5 ml olive oil
Remark: method: after 24 h and 72 h examination
Source: Bayer AG Leverkusen
08-DEC-1992 (33)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classificat.:
Method: other: (see remarks)
Year: GLP:
Test substance:
Remark: method: 0.5 ml, semi-occlusive, clipped intact and abraded skin, 24 h exposure, observation period 7 days, scoring in accordance with the Federal Hazardous Substance Act, 21 CFR, paragraph 191.11 (1964)
Source: Bayer AG Leverkusen
08-DEC-1992 (31)

5.2.2 Eye Irritation

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of
Animals:
Result: slightly irritating
EC classificat.:
Method: other: (see remarks)
Year: GLP:
Test substance: other TS: undiluted
Remark: method: 0.1 ml in the conjunctival sac of the right eye of each of 3 rabbits, 24 h exposure, then eyes rinsed with warm isotonic saline solution, observation period 5 days, the

data were scored according to the method of Draize et al. (1944)
Source: Bayer AG Leverkusen
08-DEC-1992 (29)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: slightly irritating
EC classificat.:
Method: other: (see remarks)
Year: GLP:
Test substance:
Remark: method: 0.1 ml in the conjunctival sac, observation period 7 days, scoring in accordance with the Federal Hazardous Substance Act, 21 CFR, paragraph 191.12 (1964)
Source: Bayer AG Leverkusen
08-DEC-1992 (31)

5.3 Sensitization

Type: Guinea pig maximization test
Species: guinea pig
Number of Animals:
Vehicle:
Result: sensitizing
Classification:
Method:
Year: GLP:
Test substance: other TS: 6PPD in olive oil or vaseline
Remark: 50 % sensitization (challenge with 0.05 %), 90 % sensitization (challenge with 0.5 %)
Source: Bayer AG Leverkusen
08-DEC-1992 (33)

Type: Patch-Test
Species: human
Concentration: Induction 50 %
Number of Animals: 50
Vehicle:
Result:
Classification:
Method: other: Modified Draize
Year: GLP:
Test substance: other TS: PPD; purity not stated
Remark: PPD was patch tested on 50 human volunteers at a concentration of 50% w/v in dimethylphthalate. 5 of the 50 subjects showed skin reactions during the 3-week induction phase of the study.

5 of 50 subjects showed skin reactions in the challenge phase.
20-NOV-2001 (34)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method: other: Repeated Insult Patch Test
Year: GLP:
Test substance: other TS: a 0.1 % W/V solution in dimethylphthalate
Remark: 0/50 volunteers had a positive test result
Source: Bayer AG Leverkusen
08-DEC-1992 (35)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS
Remark: 0/50 (for each rubber sample) human subjects not previously exposed to test rubber formulations had a positive patch test result
Source: Bayer AG Leverkusen
Test substance: 2 parts 6-PPD per hundred parts rubber, unvulcanized
2 parts 6-PPD per hundred parts rubber, vulcanized
20-MAY-1992 (36)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS: a rubber sample with 6PPD as additive
Remark: 17/50 subjects showed a positive reaction after challenge
Source: Bayer AG Leverkusen
08-DEC-1992 (37)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS: a rubber sample with 2 parts 6PPD per hundred parts rubber
Remark: 2/4 volunteer subjects who had reacted to previous rubber samples, had a positive patch test result
Source: Bayer AG Leverkusen
08-DEC-1992 (38)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS: a rubber sample with 2 parts 6PPD per hundred parts rubber
Remark: 5/10 volunteer subjects who had reacted to previous rubber samples, had a positive patch test result
Source: Bayer AG Leverkusen
08-DEC-1992 (39)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS: a rubber sample with 6PPD as additive
Remark: 3/10 volunteer subjects, all of whom had been previously sensitized to a rubber sample, had a positive patch test result
Source: Bayer AG Leverkusen
08-DEC-1992 (40)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS: samples with 1, 2 and 3 parts 6PPD per hundred parts rubber
Remark: 9/10 (for each rubber sample) volunteer subject who had reacted to previous rubber samples, had a positive patch test result
Source: Bayer AG Leverkusen
08-DEC-1992 (41)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS: a rubber sample with 6PPD as additive
Remark: 4/50 subjects showed a positive reaction after challenge
Source: Bayer AG Leverkusen
08-DEC-1992 (42)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS: a rubber sample with 2 parts 6PPD per hundred parts rubber
Remark: 0/50 volunteer subjects, not previously associated with either chemical had a positive patch test result
Source: Bayer AG Leverkusen
08-DEC-1992 (43)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS: 1 % Santoflex 13 in petrolatum
Remark: No skin reactions were noted in a 6-week study on 94 human volunteers. The induction phase consisted of the application of 1% 6PPD in petrolatum to the same site, 3x/week for three weeks. In the challenge phase, the test article was applied at a previously unpatched site.
20-NOV-2001 (44)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS: 50 % w/v Santoflex 13 in dimethylphthalate
Remark: 50 human volunteers were patch tested with 50 % w/v Santoflex 13 in dimethylphthalate; five of the 50 individuals showed reactions in the 3-week induction phase and 5 of 50 showed reactions in the challenge phase
Source: MonsantoBayer AG Leverkusen
31-MAY-1994 (45)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: no data
Remark: 6/9 contact dermatitis patients showed a positive reaction with 6PPD
Source: Bayer AG Leverkusen
17-AUG-1998 (46)

Type: Patch-Test
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: no data
Remark: 6/135 contact dermatitis patients showed a positive reaction with 6PPD
Source: Bayer AG Leverkusen
17-AUG-1998 (47)

Type: no data
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance: other TS: 2 % in lanolin
Remark: 15/15 IPPD-allergic patients were positive in the test with 6-PPD
Source: Bayer AG Leverkusen
08-DEC-1992 (33)

Type: other: (see remarks)
Species: guinea pig
Number of Animals:
Vehicle:
Result: not sensitizing
Classification:
Method: other: (see remarks)
Year: GLP:
Test substance:
Remark: method: application daily for 20 days (50 % paste), back, for the challenge different concentrations 10, 20, 30, 50 and 100 %) were applied to new areas of the back (no further data available)
Source: Bayer AG Leverkusen
08-DEC-1992 (30)

5.4 Repeated Dose Toxicity

Species:	rat Sex: male/female
Strain:	Sprague-Dawley
Route of admin.:	oral feed
Exposure period:	13 w
Frequency of treatment:	daily
Post. obs. period:	no data
Doses:	250, 1000 or 2500 ppm (19, 75 or 188 mg/kg b.w./d)
Control Group:	yes, concurrent no treatment
NOAEL:	250 ppm
Method:	other: EHL Protocol 85087 Ref: Multiple Comparison Procedure for Comparing Several Treatments with a Control (1955)
Year:	GLP: yes
Test substance:	other TS: Santoflex 13 Lot#KE06-121, purity: 97.1%
Result:	Santoflex 13 was administered in feed to groups of 6 week old male and female rats at the above levels. Analyses via GC verified feeding levels of 0, 230, 950 and 2300 ppm. All animals survived the length of the study. Signs of toxicity during the study were limited to reduced feed consumption/body weight gain in the high-dose males and females and mid-level males. Anemia, lymphocytopenia and thrombocytosis were present in males and females, primarily at the two highest dose levels. Increases in total bilirubin in males, and total protein, albumin, globulin, calcium and/or cholesterol in both sexes were noted in high and some mid-dose level animals. Increased liver weights were observed at the two highest dose levels. There were no gross or microscopic lesions attributed to consumption of the test material. Females at low dose levels exhibited mild anemia at the interim sampling period, but all recovered by the end of the study. Therefore, the NOEL was considered to be 250 ppm.
Reliability:	(1) valid without restriction GLP guideline study
Flag:	Critical study for SIDS endpoint

20-NOV-2001 (48) (49)

Species:	rat Sex: male/female
Strain:	Sprague-Dawley
Route of admin.:	inhalation
Exposure period:	4 w (20 exposures)
Frequency of treatment:	6 h/d
Post. obs. period:	no data
Doses:	0.054, 0.236 or 0.477 mg/l
Control Group:	yes, concurrent no treatment
Method:	other: Subacute Dust Inhalation Study IBT #8562-09721 (Audited)
Year:	GLP: yes
Test substance:	other TS: Santoflex 13 Powder Lot #KD03-017, purity: 97.1%
Result:	4 groups of 5 male and 5 female young adult albino rats were exposed to either zero, low, intermediate or high dust concentrations of the test article. Test dusts were suspended in streams of clean, dry air, and introduced through the top center of exposure chambers and exhausted out the bottom. GC analytical testing confirmed concentrations and total weight of test dusts. All but one animal survived until sacrifice on Day 28. Hypoactivity was noted in all test groups. Mid and high-dose animals exhibited swollen snouts and scratching. Mean body weights of treated animals compared favorably with those of controls. Results of gross necropsy indicated increased liver and kidney weights of treated animals over those of controls. Lung weights were reduced in high-dose makes and mid-dose females. Mid-dose treated males exhibited increased spleen weights. No significant differences were noted in the weights of the brains, gonads and hearts of treated animals when compared to controls. No gross or histopathologic alterations attributed to the test article were observed in any of the treated animals. Mean corpuscular hemoglobin was reduced in high-dose males; elevations in SGPT and lowered glucose levels in mid- and high-dose males were correlated with increased relative liver weights; no treatment related gross lesions were noted at necropsy.
Reliability:	(1) valid without restriction GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag:	Critical study for SIDS endpoint

20-NOV-2001 (50)

Species:	rat Sex: male/female
Strain:	other: Charles River CD
Route of admin.:	oral feed
Exposure period:	24 months
Frequency of treatment:	daily
Post. obs. period:	no
Doses:	100, 300, 1000 ppm (8, 23, 75 mg/kg b.w./day)
Control Group:	yes, concurrent no treatment
NOAEL:	23 mg/kg
LOAEL:	75 mg/kg
Method:	other: 2-Year Chronic Oral Toxicity IBT Protocol # 622-05400A (1974)
Year:	GLP: yes
Test substance:	other TS: 6PPD. Powder, purity: 96.9%
Remark:	hematology, clinical chemistry and urinalysis were conducted at 3, 6, 12 and 24 months, the calculation of the dose levels is based on 1 ppm corresponds to 0.075 mg/kg b.w.; 50 male and 50 female rats per group.
Result:	6PPD was fed at the above doses to groups of 200 male and 200 female rats over a two-year period, beginning when the males were 28 days old and the females 29 days old. Dose levels were verified by GC analysis. Body weight, food consumption, behavior, hematology, blood chemistry and urinalysis results were recorded throughout the study. Complete gross necropsies were conducted on all animals found dead, on all animals sacrificed in extremis, and on all remaining animals at 24 months. All organs or tissues with grossly visible lesions were submitted for histologic examination. Statistical reductions in body weight were noted in high-dose males during Weeks 1-5. High-dose females exhibited statistically reduced body weights throughout the study. Body weights and weight gain of the mid- to low-dose animals compared favorably to controls. Frequency and distribution of deaths during the study were similar between treated animals and controls. Gross pathological examination of animals that died during the study did not reveal any relation to death and the test article. There were no unusual behaviors noted in test animals during the study. A significant reduction in erythrocyte counts was noted in high-dose males at 3 months and in high-dose females at 3, 6, and 9 months. However, the same animals had erythrocyte counts similar to controls at all subsequent blood collections. Hemoglobin concentration, while still considered to be within normal range, was statistically reduced for high-dose males at 3, 12 and 18 months. High-dose females exhibited similar reductions at 6, 12 and 18 months. Hematocrit values among high-dose animals were significantly lower than controls, and were at the lower limits at 3 and 12 months for males, and 3,6 and 12 months for females. Hematocrit values in these animals exhibited a slight increase at 18 and 24 months. Urinalysis studies, which included monitoring of glucose, albumin, microscopic elements, pH and specific gravity, were similar for both treated and control

groups throughout the study. Gross pathological examination of animals sacrificed at 24 months revealed similar findings for both treated and control groups. Statistical analysis of absolute organ weights, organ to body weight ratios and organ to brain weight ratios compared favorably across the test and control groups, and were within the range of expected values for albino rats of this age and strain. Histopathological examination of organs and tissue taken from high-dose animals and controls at 24 months revealed no treatment-related lesions. Any lesions noted were from those of naturally-occurring diseases, and were noted in both populations. Microscopic examination of suspect lesions from all sacrificed animals and also those that died during the study. No differences were noted between test and control rats as to the organ system involved, type or classification of neoplasms..

Reliability: (1) valid without restriction
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint

20-NOV-2001 (51) (52)

Species: rat Sex: male/female
Strain: no data
Route of admin.: oral feed
Exposure period: after 12 months interim sacrifice (no further data)
Frequency of treatment: daily
Post. obs. period: no data
Doses: 50, 250 or 1500 ppm (4, 20 or 120 mg/kg bw/d)
Control Group: yes
Method:
Year: GLP: no data
Test substance: other TS: Santoflex 13
Remark: The NOEL for chronic toxicity was determined to be 50 ppm, and a NOEL for oncogenic effects was determined to be at least 1500 ppm
Result: decreased body weights in mid- and high-exposure females and high-exposure males; various hematological changes in mid- and high-exposure females and high-exposure males; some high-exposure male and female serum chemistry alterations (increased cholesterol, total protein, globulin and calcium); absolute and relative liver weights were increased for mid-exposure male rats at study termination and for high-exposure male and female rats after one year of exposure and at the end of the study; histopathological examination revealed pigment in the hepatocytes and reticuloendothelial cells of high-exposure females; mean absolute and relative kidney weights were also statistically significantly increased for high-exposure males and females compared to controls at the 12-month interim sacrifice only; a slight increase in the severity but not the incidence of chronic nephropathy was noted for high-expo-

sure males and females compared to controls at both interim and terminal sacrifice periods; high exposure males demonstrated increased absolute and relative spleen weights compared to controls at the 12-month exposure period only; neoplastic findings were similar between control and Santoflex 13-treated animals

Source: MonsantoBayer AG Leverkusen

31-MAY-1994 (53)

Species: rat Sex: no data
Strain:
Route of admin.: gavage
Exposure period: 24 days
Frequency of treatment: once a day
Post. obs. period: no data
Doses: 250 mg/kg b.w./day for the first 4 days, thereafter being increased 50 % every 5 days, no further data available
Control Group: yes
Method:
Year: GLP:
Test substance:
Result: no death, body weight gain within the normal range, increased oxygen consumption, suppression of the central nervous system and of the synthezising function of the liver (content of hippuric acid in a 24 h urine sample was decreased), decreased ascorbic acid content in the liver

Source: Bayer AG Leverkusen

08-DEC-1992 (30)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium TA-1535 TA-1537 TA-1538 TA-98 TA-100
Concentration: 0.001, 0.01, 0.1, 1.0 and 5.0 micrograms/plate
Cytotoxic Conc.:
Metabolic activation: with and without
Result: negative
Method: other: Ames Plate Test (Overlay method) 1975; OECD 471 equivalent
Year: GLP: yes
Test substance: other TS: 6PPD #BIO76-277, purity: >96%
Remark: Stock solutions prepared in DMSO. No evidence of mutagenic activity in any assay conducted with or without activation using the S-9 homogenate from Arochlor-induced rat livers.
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint

20-NOV-2001 (54)

Type: Ames test
System of testing: Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100
Concentration: 0.167, 0.500, 1.67, 5.00, 16.7 and 50.0 micrograms/plate
Cytotoxic Conc.: Precipitation conc: >500 micrograms/plate
Metabolic activation:
Result:
Method: other: Revised Method for the Salmonella Mutagenicity Test (1983), Maron, D.M. and Ames, B.N.
Year: GLP: yes
Test substance: other TS: 6PPD purple solid #4065461, purity: >96%
Remark: Stock solutions prepared in DMSO. All tester strains contained a uvrB deletion mutation and an rfa mutation. Cytotoxicity of test article was determined in a screening test on duplicate cultures of TA1538 and TA100 in the absence of S9. In the definitive assay, inhibited growth was observed at concentrations >5.00, both with and without S9 activation. Revertant frequencies for all doses, in all strains, both with and without metabolic activation were equal to or less than those of controls. Results for the test article were negative under the test conditions.
Reliability: (1) valid without restriction
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
20-NOV-2001 (55)

Type: Gene mutation in Saccharomyces cerevisiae
System of testing: Saccharomyces cerevisiae D4
Concentration: 0.001, 0.01, 0.1, 1.0 and 5.0 micrograms/plate
Cytotoxic Conc.:
Metabolic activation: with and without
Result: negative
Method: other: Ames Plate Test (Overlay method) 1975; OECD 471 equivalent
Year: GLP: yes
Test substance: other TS: 6PPD #BIO76-277, purity: >96%
Remark: Stock solutions prepared in DMSO. No evidence of mutagenic activity in any assay conducted with or without activation using the S-9 homogenate from Arochlor-induced rat livers.
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
20-NOV-2001 (54)

Type: Mammalian cell gene mutation assay
System of testing: Mouse lymphoma cells (L5178Y TK+/-)
Concentration: 0.25, 0.5, 1.0, 2.0, 4.0 or 8.0 micrograms/ml
Cytotoxic Conc.: With metabolic activation: 33 micrograms/ml; Without metabolic activation: > 4 micrograms/ml
Metabolic activation: with and without
Result: negative
Method: other: OECD 476 equivalent
Year: GLP: yes
Test substance: other TS: 6PPD/CP22423 , purity: >96%
Remark: Negative for ability to induce forward mutations at the TK locus.
Reliability: (1) valid without restriction
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
20-NOV-2001 (56)

Type: Mammalian cell gene mutation assay
System of testing: Chinese hamster ovary cells (CHO/HGPRT)
Concentration: up to 5 ug/ml without S9-mix, up to 15 ug/ml with S9-mix
Cytotoxic Conc.: With metabolic activation: 9 micrograms/ml; Without metabolic activation: 4 micrograms/ml; Solubility limit of test article = 333 micrograms/ml
Metabolic activation: with and without
Result: negative
Method: other: CHO/HGPRT Mutation Assay (1979) Hsie, et.al.
Year: GLP: yes
Test substance: other TS: 6PPD purple pellets lot# KH04, purity: 96%
Remark: 6PPD was tested in CHO cells at different S9 concentrations up up to cytotoxic concentrations in two range-finding, one initial and one confirmatory experiments. The cytoxicity of the test article decreased with increasing S9 concentrations. No statistically significant mutagenicity was observed. 6PPD is not considered mutagenic to CHO cells under test conditions.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
20-NOV-2001 (57)

Type: Unscheduled DNA synthesis
System of testing: primary rat hepatocytes
Concentration: 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000 and 5000 micrograms/ml
Cytotoxic Conc.: 50 micrograms/ml
Metabolic activation: without
Result: negative
Method: other: Williams, G.M., 1977. Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures
Year: GLP: yes
Test substance: other TS: 6PPD purple pastilles Lot# KH04-70, purity: 96%
Remark: Reagent grade Acetone (1%) as solvent. 6PPD was examined for genotoxicity in the UDS Assay. Primary rat lever cell cultures used for both the preliminary and replicate experiments were derived from the livers of two adult male Fischer-344 rats (13 and 18 weeks old, respectively).
Quantitative autoradiographic grain-counting was performed using an ARTEK Model 980 colony counter interfaced with a Zeiss Universal Microscope via an ARTEK TV camera. Data were fed directly to a VAX computer. Cytotoxicity was observed at concentrations of 50 micrograms/ml and above in both the preliminary and replicate experiments. UDS was measured at concentrations of the test article between 0.1 and 10 micrograms/ml in both experiments. The net grain counts were negative at each concentration of the test compound, in the solvent control, and in the medium control, in contrast to the strong positive response produced in both experiments by the positive control. These results indicate that 6PPD is not a genotoxic agent under the conditions of the in vitro rat hepatocyte DNA repair assay.
Reliability: (1) valid without restriction
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
20-NOV-2001 (58)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells (CHO)
Concentration:
Cytotoxic Conc.:
Metabolic activation: no data
Result: negative
Method: other: chromosomal aberrations
Year: GLP:
Test substance:
Remark: no further data available
Source: Bayer AG Leverkusen
20-NOV-2001 (59)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: up to 15 ug/ml
Cytotoxic Conc.:
Metabolic activation: no data
Result:
Method:
Year: GLP: no data
Test substance: other TS: Santoflex 13
Remark: effects: Santoflex 13 showed a marginal potential for inducing chromosomal aberrations
type: chromosomal aberration assay
Source: MonsantoBayer AG Leverkusen
20-NOV-2001 (60)

Type: Ames test
System of testing: Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Concentration: up to 1000 ug/plate
Cytotoxic Conc.:
Metabolic activation: with and without
Result: negative
Method: other: Ames Salmonella/Microsome (EPA/OECD)
Year: 1984 GLP:
Test substance: other TS: Flexzone 7F
Source: Bayer AG Leverkusen
Reliability: (2) valid with restrictions
21-OCT-1999 (61)

Type: Ames test
System of testing: Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Concentration:
Cytotoxic Conc.:
Metabolic activation: with and without
Result: negative
Method:
Year: GLP:
Test substance:
Remark: no further data available
Source: Bayer AG Leverkusen
08-DEC-1992 (62)

Type: Ames test
System of testing: Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537
Concentration: up to 200 ug/plate
Cytotoxic Conc.:
Metabolic activation: with and without
Result: negative
Method:
Year: GLP:
Test substance:
Source: Bayer AG Leverkusen
08-DEC-1992 (63)

Type: Ames test
System of testing: Salmonella typhimurium
Concentration:
Cytotoxic Conc.:
Metabolic activation:
Result: negative
Method:
Year: GLP:
Test substance:
Remark: no further data available
Source: Bayer AG Leverkusen
08-DEC-1992 (64) (65) (66)

Type: Ames test
System of testing: Salmonella typhimurium (no further data)
Concentration: up to 500 ug/plate
Cytotoxic Conc.:
Metabolic activation: with and without
Result: negative
Method:
Year: GLP: no data
Test substance: other TS: Santoflex 13
Source: MonsantoBayer AG Leverkusen
31-MAY-1994 (67)

Type: Mammalian cell gene mutation assay
System of testing: Chinese hamster ovary cells (CHO/HGPRT)
Concentration: up to 0.6 ug/ml without S-9 mix, up to 55 ug/ml with S-9 mix
Cytotoxic Conc.:
Metabolic activation: with and without
Result: negative
Method:
Year: GLP:
Test substance:
Source: Bayer AG Leverkusen
08-DEC-1992 (68)

Type: Mitotic recombination in Saccharomyces cerevisiae
System of testing: Saccharomyces cerevisiae D4
Concentration: no data
Cytotoxic Conc.:
Metabolic activation: no data
Result: negative
Method:
Year: GLP: no data
Test substance: other TS: Santoflex 13
Source: MonsantoBayer AG Leverkusen
31-MAY-1994 (69) (70)

Type: Sister chromatid exchange assay
System of testing: Chinese hamster ovary cells (CHO)
Concentration:
Cytotoxic Conc.:
Metabolic activation: no data
Result: negative
Method:
Year: GLP:
Test substance:
Remark: no further data available
Source: Bayer AG Leverkusen
08-DEC-1992 (59)

Type: Unscheduled DNA synthesis
System of testing: primary rat hepatocyte
Concentration: up to 1000 ug/well
Cytotoxic Conc.:
Metabolic activation: without
Result: negative
Method:
Year: GLP:
Test substance:
Source: Bayer AG Leverkusen
08-DEC-1992 (71)

Type: Unscheduled DNA synthesis
System of testing: primary rat hepatocytes
Concentration: up to 1000 ug/ml
Cytotoxic Conc.:
Metabolic activation:
Result: negative
Method:
Year: GLP: no data
Test substance: other TS: Flexzone 7F
Source: MonsantoBayer AG Leverkusen
31-MAY-1994 (72)

5.6 Genetic Toxicity 'in Vivo'

Type: Cytogenetic assay
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: gavage
Exposure period: 6, 18 and 30 hours
Doses: 1000 mg/kg bw
Result: negative
Method: other: EPA Health Effects Test Guidelines EPA 560/6-82-09
Year: 1984 GLP: yes
Test substance: other TS: 6PPD Lot# KJ09-165, purity: 96%
Remark: Not clastogenic under test conditions. Mild to severe pharmacotoxic effects observed in test animals indicated that the test article was administered near the maximum tolerated dose.
Reliability: (1) valid without restriction
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
20-NOV-2001 (73)

Type: Cytogenetic assay
Species: mouse Sex: male
Strain:
Route of admin.: i.p.
Exposure period: twice within 24 hours
Doses: 100 and 200 mg/kg bw
Result: negative
Method: other: no data
Year: GLP: no data
Test substance: no data
Result: no induction of chromosomal abnormalities
Source: Bayer AG Leverkusen
20-NOV-2001 (74)

Type: Micronucleus assay
Species: mouse Sex: male/female
Strain: CD-1
Route of admin.: i.p.
Exposure period: 1 day
Doses: 1000 mg/kg
Result: negative
Method:
Year: GLP:
Test substance:
Remark: clinical signs were assessed
Result: no increased number of micronucleated erythrocytes
Source: Bayer AG Leverkusen
20-NOV-2001 (75) (76)

Type: Micronucleus assay
Species: mouse Sex: male
Strain:
Route of admin.: i.p.
Exposure period: twice within 24 hours
Doses: 100, 150 and 200 mg/kg bw
Result: negative
Method: other: no data
Year: GLP: no data
Test substance: no data
Result: no induction of micronucleated erythrocytes in bone marrow
Source: Bayer AG Leverkusen
20-NOV-2001 (74)

5.7 Carcinogenicity

Species: rat Sex: male/female
Strain: other: Charles river CD
Route of admin.: oral feed
Exposure period: 24 months
Frequency of treatment: daily
Post. obs. period: no
Doses: 100, 300, 1000 ppm (8, 23, 75 mg/kg b.w./day)
Result:
Control Group: yes, concurrent no treatment
Method:
Year: GLP:
Test substance:
Remark: the calculation of the dose levels is based on 1 ppm corresponds to 0.075 mg/kg b.w.; 50 male and female rats per group
Result: the number and type of neoplastic and nonneoplastic lesions were comparable between groups
Source: Bayer AG Leverkusen
08-DEC-1992 (77)

Species: rat Sex: male/female
Strain: no data
Route of admin.: oral feed
Exposure period: after 12 months interim sacrifice (no further data)
Frequency of treatment: daily
Post. obs. period: no data
Doses: 50, 250 or 1500 ppm (4, 20 or 120 mg/kg bw/d)
Result:
Control Group: yes
Method:
Year: GLP: no data
Test substance: other TS: Santoflex 13
Remark: a NOEL for oncogenic effects was determined to be at least 1500 ppm
Result: neoplastic findings were similar between control and Santoflex 13-treated animals (no further data)
Source: MonsantoBayer AG Leverkusen
31-MAY-1994 (53)

Species: other: (see remarks) Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses:
Result:
Control Group:
Method:
Year: GLP: yes
Test substance:
Remark: BALB/3T3 cells; cell transformation assay under nonactivation conditions
Result: negative
Source: Bayer AG Leverkusen
08-DEC-1992 (78)

5.8 Toxicity to Reproduction

Type: Fertility
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: gavage
Exposure Period: Males: 42 or 49 days, Females: 14 days prior to mating through Day 7 of gestation
Frequency of treatment: daily
Premating Exposure Period
male: 28 days.
female: 14 days
Duration of test:
Doses: 0, 40, 200 or 1000 ppm
Control Group: yes, concurrent vehicle
NOAEL Parental: > 1000 ppm
NOAEL F1 Offspr.: > 1000 ppm
Method: other: Fertility Study and Early Embrionic Development to Implantation in Rats, DRL
Year: 1998 GLP: no data
Test substance: other TS: CD-13, purity >98%
Remark: The test article is being evaluated as a new diagnostic drug of Helicobacter pylori. To this end, several reproductive and developmental toxicity studies have been conducted recently by this laboratory. All reports published to date have indicated that there are no reproductive, developmental or fetotoxic effects of this chemical under the test conditions.
Result: Groups of male and female rats were dosed with the test article at the above levels prior to mating. Males and females from the same dose levels were paired. Animals were observed for body weight, weight gain, food consumption, appearance, behavior, copulation index and fertility index during the life phase of the study. Mated females were

sacrificed on Day 14 of gestation and the fetuses removed via Cesarean Section. Fetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies as well as developmental variation
General parental toxicity: All animals survived until planned sacrifice. There were no effects of treatment observed on mean body weight, weight gain, appearance, behavior, physical viability, copulation index or fertility index. There were no remarkable findings in gross necropsy or organ weights.
Toxicity to offspring: The number of corpora lutea and implantations, implantation rate, fetal mortality, and number of live fetuses were not affected by the test article.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
20-NOV-2001 (79)

Type: other: Three generation study
Species: rat Sex: male/female
Strain: other: Charles river CD
Route of admin.: oral feed
Exposure Period: for three successive generations
Frequency of treatment: daily
Duration of test:
Doses: 100, 300, 1000 ppm (8, 23, 75 mg/kg b.w./day)
Control Group: yes, concurrent no treatment
NOAEL Parental: 10 ppm
Method: other: the F0-generation received the test compound for 11 weeks before mating and during mating, gestation and lactation for two successive litters (F1a, F1b)
Year: GLP:
Test substance:
Remark: the calculation of the dose levels is based on 1 ppm corresponds to 0.075 mg/kg b.w.
Result: F0-generation: no effect on fertility, no effect on behaviour, reduced body weight gain at the mid and high dose levels, no substance-related histopathological effects
F1-generation, F2-generation, F3-generation: no effect on fertility, no effect on behaviour, no substance-related histopathological effects
Source: Bayer AG Leverkusen
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
20-NOV-2001 (80) (52)

Type: other: rangefinding study
Species: rat Sex: female
Strain: no data
Route of admin.: gavage
Exposure Period: gestation days 6 to 15
Frequency of treatment: daily
Duration of test:
Doses: 100, 300, 600, 1000 or 2000 mg/kg bw/d
Control Group: yes
Method:
Year: GLP: no data
Test substance: other TS: Santoflex 13
Result: excessive toxicity was noted at 600 mg/kg bw/d and above; intrauterine survival was not affected by treatment at 100 or 300 mg/kg bw/d
Source: MonsantoBayer AG Leverkusen
31-MAY-1994 (81)

5.9 Developmental Toxicity/Teratogenicity

Species: rat Sex: female
Strain: Sprague-Dawley
Route of admin.: gavage
Exposure period: days 6-15 of gestation
Frequency of treatment: daily
Duration of test: 20 days
Doses: 0, 50, 100 or 250 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Maternalt.: = 50 mg/kg bw
NOAEL Teratogen.: > 250 mg/kg bw
Method: other: Teratology - Principles and Techniques, J.G. Wilson 1965
Year: GLP: yes
Test substance: other TS: 6PPD Lot# KE-10-143 purity: >97%
Remark: Four groups of 25 bred female rats were dosed with the test article at 0, 50, 100 and 250 mg/kg/body weight. Dosages were determined in a preceding range-finding study. Survival was 100% in all groups. Throughout gestation, all animals were observed 2x/day for appearance, behavior, body weight and food consumption. On Day 20, all test animals were sacrificed and the fetuses removed via Cesarian Section. Fetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies as well as developmental variation.
This was a follow-up study to a range-finding study (Monsanto WI-85-304) that noted excessive maternal toxicity at dose levels of 2000, 1000 and 600 mg/kg/day, with clinical signs of toxicity in the 300 mg/kg/day group. Intrauterine survival was not affected at the 100 and 300 mg/kg/day dose levels.
Result: Maternal general toxicity: Clinical signs noted in the Mid- to High-dose groups included salivation prior to dosing, soft stool, diarrhea and green fecal discoloration. Maternal body weights and weight gain were comparable in all groups. No

morphopathological changes which could be attributed to the test article were observed in any of the treated animals
Pregnancy/litter data: No abortions or premature deliveries occurred in any test group.
Foetal data: No differences that could be associated with the test article were observed between the control group and the treated groups with respect to number of viable fetuses, early and late resorptions, fetal sex ratios or fetal weights. The types of malformations and the frequency of such mutations occurring during this study were not those indicative of a teratogenic response. There was a small, non-statistically significant increase in the incidence and number of skeletal variations in the treated groups. However, these were judged to be common developmental variations of this species and have been observed to occur with similar incidence in the historical data.
Not teratogenic or embryo/fetotoxic under test conditions.

Reliability: (1) valid without restriction
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint

20-NOV-2001 (82)

Species: rabbit Sex: female
Strain: other: New Zealand
Route of admin.: oral unspecified
Exposure period: gestation day 6 through day 18 inclusive
Frequency of treatment: once a day
Duration of test: post observation: sacrifice on gestation day 29
Doses: 10, 30 mg/kg b.w./day
Control Group: other: yes, empty gelatin capsules
NOAEL Maternalt.: 30 mg/kg bw
Method:
Year: GLP:
Test substance: other TS: Santoflex 13
Remark: in a pilot study 100 and 300 mg/kg b.w./day caused maternal toxicity
Result: maternal body weight loss and mortality comparable to the controls, no treatment related gross lesions were noted at necropsy; a slight increase in the number of resorption sites per 100 implantation sites for the 30 mg/kg b.w. group (38.6 %) when compared to the controls (31.4 %), the number of live young per 100 implantation sites for the 10 mg/kg b.w. group (48.3 %) and for the 30 mg/kg b.w. group (38.6 %) were moderately decreased when compared to the controls (68.6 %); no increase in the incidence of external, visceral and skeletal abnormalities
Source: Bayer AG Leverkusen

20-NOV-2001 (83)

Species: Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method: other: test compounds were tested for embryotoxicity and induction of malformations in three-day chicken embryos
Year: GLP:
Test substance:
Result: slight effects
Source: Bayer AG Leverkusen
08-DEC-1992 (84) (85)

5.10 Other Relevant Information

Type: other
Remark: A comprehensive description of the toxicity profile is available in the BUA-Report
Source: Bayer AG Leverkusen
12-NOV-1998 (86)

Type:
Remark: Revision date: August, 1998
Source: Bayer AG Leverkusen
17-AUG-1998

5.11 Experience with Human Exposure

Memo: Occupational eczema study - 6PPD and IPPD exposures
Remark: Cross sensitization in rubber workers exposed to various members of the PPD family have been reported. Anecdotal evidence suggests that this class of compounds has a high potential for skin sensitization with prolonged and repeated exposures of sensitive individuals.
20-NOV-2001 (87)

Remark: In the rubber industry 6PPD was detected in the urine of 6PPD exposed workers
Source: Bayer AG Leverkusen
08-DEC-1992 (88)

Remark: analytical methods for the determination of the trace levels of 6PPD in human urine are described (in the publication of Pavan et. al the abbreviation 6PPD is used however the substance is called N-(2,3-dimethylpropyl)-N-phenyl-1,4-benzenediamine with the CAS-No. 739-24-8)
Source: Bayer AG Leverkusen
08-DEC-1992 (89) (90)

(1) ASTM D-1519 / Flexsys Physical Methods of Analysis

(2) Hawley, G.G.: The Condensed Chemical Dictionary, 9th ed., 672 (1977)

(3) Safety data sheet Bayer AG

(4) Flexsys Physical Methods of Analysis, FF97.8-1 Flexsys Standard Method 1997

(5) Bayer AG data, 1997

(6) Meylan, W.M. and. P.H. Howard, 1995 J. Pharm. Sci. 84: 83-92

(7) Calculation Bayer AG (1992)

(8) Tests Monsanto, cited in Monsanto Material Safety Data Sheet Santoflex 13, Antioxonant, 4186

(9) Monsanto ES-78-SS-20 MIC Environmental Science Dec. 1978

(10) Monsanto Study K-45.

(11) Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

(12) Monsanto #32304. Analytical BioChemistry Labs. March, 1986

(13) Monsanto study ES-78-SS28

(14) Monsanto report MO-93-9110 (ES-93-SS-05)

(15) Syracuse Research Corporation

(16) Monsanto ES-81-SS-52 Environmental Sciences Labs Dec. 1981

(17) Study Bayer AG, WV-LE Umweltschutz, AWALU, 1984

(18) Simpson, K.E., Int. Biodeterioration 24, 307-312 (1988)

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7.1 End Point Summary

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7.2 Hazard Summary

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7.3 Risk Assessment

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Substituted p-Phenylenediamines Category – Comments of Environmental Defense

(Submitted via Internet May 15, 2002)

Environmental Defense appreciates this opportunity to submit comments on the robust summary/test plan for the Substituted p-Phenylenediamines Category.

In its Test Plan for the substituted p-phenylenediamine category, the Rubber and Plastic Additives (RAPA) Panel of the American Chemical Council proposes that a number of antioxidants based on p-phenylenediamine and having similar structural, physicochemical, and toxicological characteristics be considered as a category. The Test Plan and Robust Summaries submitted for these chemicals are well organized, and the data are clearly presented and sufficiently complete to support interpolation and/or extrapolation to assess data gaps where they exist. Background material presented in the Test Plan also indicates that these chemicals are used only in finished products, and thus there would appear to be limited chance of environmental or consumer exposure. The Test Plan is supported by numerous, well-designed and clearly described studies. Most of these studies were conducted under GLP. Further, all available data for the parent p-phenylenediamine and substituted p-phenylenediamines described here as well as in other literature reviewed indicate these compounds are negative carcinogens when tested in animals. Therefore, we support consideration of the p-phenylenediamines as a category and agree that no additional testing is necessary. Our specific comments are limited to the following:

1. An earlier Test Plan/Robust Summary submitted by RAPA for the Substituted Diphenylamines included considerable data for the base chemical diphenylamine. Similar data for the base chemical of the substituted p-phenylenediamines, p-phenylenediamine, are not included in the present Test Plan/Robust Summary. Since p-phenylenediamine and it is a likely degradation and/or metabolic product of the substituted phenylenediamines we believe this is an omission of critical data. That is, the carbon-nitrogen bond of the p-phenylenediamines is much weaker than the carbon-carbon bond of the diphenylamines making p-phenylenediamine a likely degradation and/or metabolic product of compounds in this category. p-Phenylenediamine has been the subject of considerable research, and including discussion of it would further enhance a thorough submission.

2. Data in the Robust Summaries indicate that these chemicals hydrolyze rapidly. The data do not, however, describe the hydrolysis products. As discussed above, a likely hydrolysis product would be p-phenylenediamine, thus providing a second reason to list data for this chemical.

3. The substituted p-phenylenediamines hydrolyze rapidly in pure water, but degrade very slowly in aqueous sludge. Thus, they may be tightly adsorbed onto the organic matter in sludge and could thus persist in the environment to possibly result in bioaccumulation and toxicity to aquatic organisms if they should somehow be released. This possibility should be addressed.

4. Some members of the category are irritating or corrosive to skin but are otherwise of relatively low toxicity to mammals. That fact was not made obvious in the Test Plan and should be addressed. RAPA may also want to point out that some of the toxicity observed in mammals may have resulted from gastrointestinal irritation that occurred as a result of gavage dosing, a situation that is unlikely to occur with humans.

5. No synonyms are listed for these chemicals. Each has at least one commercial synonym “Santoflex #, etc.” It would be helpful to include a list of these synonyms.

Thank you for this opportunity to comment.

Hazel B. Matthews, Ph.D.
Consulting Toxicologist, Environmental Defense

Karen Florini
Senior Attorney, Environmental Defense

November 15, 2002

Dr. Anne P. LeHuray
Technical Contact
The American Chemistry Council's Rubber and Plastic
Additives Panel
1300 Wilson Boulevard
Arlington, VA 22209

Dear Dr. LeHuray:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for the Substituted p-Phenylenediamines Category posted on the ChemRTK HPV Challenge Program Web site on January 17, 2002. I commend The American Chemistry Council's Rubber and Plastic Additives Panel for their commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its Challenge Web site, EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the enclosed Comments on the HPV Challenge Web site within the next few days. As noted in the comments, we ask that The American Chemistry Council's Rubber and Plastic Additives Panel advise the Agency, within 90 days of this posting on the Web site, of any modifications to its submission.

If you have any questions about this response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-564-7649. Submit questions about the HPV Challenge Program through the "Contact Us" link on the HPV Challenge Program Web site pages or through the the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at tsca-hotline@epa.gov.

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

-S-

Oscar Hernandez, Director
Risk Assessment Division

Enclosure

cc: C. Auer
A. Abramson
W. Penberthy
M. E. Weber

EPA Comments on Chemical RTK HPV Challenge Submission:
Substituted p-Phenylenediamines

SUMMARY OF EPA COMMENTS

The sponsor, the Rubber and Plastic Additives (RAPA) Panel of the American Chemistry Council, submitted a test plan and robust summaries to EPA for the p-Phenylenediamines Category dated December 13, 2001. EPA posted the submission on the ChemRTK HPV Challenge Web site on January 17, 2002. The category consists of N,N'-di-*sec*-butyl-*p*-phenylenediamine, N,N'-bis(1,4-dimethylpentyl)-*p*-phenylenediamine, 1,4-benzenediamine, N,N'-mixed phenyl and tolyl derivatives, N-(1,4-dimethylpentyl)-N'-phenyl- *p*-phenylenediamine, and N-(1-methylheptyl)-N'-phenyl-*p*-phenylenediamine.

EPA has reviewed this submission and has reached the following conclusions:

1. Category Justification. The submitter's support for grouping the chemicals in this category with regard to toxicological properties is acceptable.

2. Physicochemical Properties and Environmental Fate. (a) A vapor pressure test needs to be conducted for N,N'-di-*sec*-butyl-*p*-phenylenediamine. (b) A biodegradation study needs to be conducted for N,N'-di-*sec*-butyl-*p*-phenylenediamine. (c) The submitter needs to address deficiencies in robust summaries for water solubility.

3. Health Effects. EPA reserves judgment on the adequacy of the submitted toxicity data pending receipt of additional information in the robust summaries.

4. Ecological Effects. EPA reserves judgment on the adequacy of the submitted toxicity data on fish, daphnia, and green algae, pending adequate explanation of test conditions (addressing deficiencies in the robust summaries) and degradation products (see item 5 below) for these studies.

5. Other issues. The submitter did not discuss essential information about environmental fate provided in one robust summary that also is a critical factor in the fate and ecotoxicity evaluation of all category members. Appropriate discussion of these areas needs to be added to the test plan.

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.

EPA COMMENTS ON THE SUBSTITUTED p-PHENYLENEDIAMINES CHALLENGE SUBMISSION

Category Definition

The submitter proposed a category defined as *p*-phenylenediamines covering five chemicals containing amino groups, which are each substituted with one alkyl or phenyl group. The submitter has subdivided the category into two groups: 1) N–alkyl substituents (N-Alkylated *p*-Phenylenediamines), and 2) N-aryl or mixed N-aryl/N-alkyl substituents (4-Aminodiphenylamine Derivatives). The N-Alkylated *p*-Phenylenediamine subcategory includes N,N'-di-*sec*-butyl-*p*-phenylenediamine (CAS no. 101-96-2) and N,N'-bis(1,4-dimethylpentyl)-*p*-phenylenediamine (CAS no. 3081-14-9) and the 4-Aminodiphenylamine Derivatives subcategory includes N,N'-mixed phenyl and tolyl derivatives of 1,4-benzenediamine (CAS no. 68953-84-4); N-(1,4-dimethylpentyl)-N'-phenyl- *p*-phenylenediamine (CAS no. 3081-01-4); and N-(1-methylheptyl)-N'-phenyl-*p*-phenylenediamine (CAS no. 15233-47-3). In addition, the submitter included

supporting data on two non-category chemicals sponsored in the OECD SIDS program: N-isopropyl-N'-phenyl-*p*-phenylenediamine (CAS no. 101-72-4) and N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine (CAS no. 793-24-8).

Category Justification

The submitter's justification for the category is based on the structural similarity of the substances and an expectation of similar physicochemical, environmental, and toxicological properties among the members. To further refine the comparison of the properties of the members, the submitter has subdivided the category into two groups to better match the classes of substituent groups. One subgroup (alkylated – PPD) contains compounds with alkyl substituents only; the other subgroup (4-aminodiphenylamine derivatives) contains either a mixture of alkyl/aryl or aryl only substituents. The submitter has provided a rationale to demonstrate similarities between the members of each subgroup for each endpoint. For the environmentally important physicochemical endpoints, ecotoxicity endpoints, and health effects endpoints, the submitter has provided sufficient data to establish a pattern for both subgroups where the values are reasonably similar.

From the standpoint of physicochemical properties, the values for two of the endpoints (*e.g.,* water solubility and octanol/water partition coefficient) are reasonably similar among the members in each subgroup. For the vapor pressure endpoint, the compounds have been shown to have low vapor pressures (with the exception of CAS No. 101-96-2, whose value is larger than expected based on both its structure and a comparison to the value reported for the other member of the alkylated N-PPD subgroup). The data for the physicochemical endpoints support the category.

The submitter demonstrates a reasonable consistency in the values of the members for the hydrolysis and photodegradation endpoints. In addition, all tested members of the category show low or virtually no biodegradation. However, important chemical fate information was omitted from the category discussion and needs to be added (see comments below under Environmental Fate).

Finally, the available environmental fate data have many illuminating consistencies across endpoints that need to be fully coordinated and discussed in the final category analysis required of the submitter.

Test Plan

Chemistry (melting point, boiling point, vapor pressure, partition coefficient, and water solubility)

Vapor Pressure. A test needs to be conducted for CAS No. 101-96-2. The submitted vapor pressure differs substantially from estimated values; the measurement was not in accord with OECD TG 104; and the estimated value is in the range where a measured value is necessary.

Environmental Fate (photodegradation, stability in Water, biodegradation, fugacity)

In the robust summary for the third stability in water study for N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine, the submitter provided a useful discussion of the fate of this type of substance in the presence of oxygen and water. The discussion is essential to evaluating all fate and ecotoxicity endpoints, and should have been included and expanded upon in the test plan discussion of these and any other relevant endpoints and studies.

Biodegradation. A ready biodegradation test needs to be conducted for CAS No. 101-96-2. While in general the compounds in this category do not appear to biodegrade, CAS No. 3081-14-9 did show

partial degradation in an inherent test. CAS No. 101-96-2 is a simpler compound with less branching and shorter carbon side chains and so has an even greater potential for biodegradation.

Stability in water. Evaluation of this endpoint is impeded because of inaccuracies in the robust summaries related to identification of degradation products, which need to be corrected (see Specific Comments on Robust Summaries).

Health Effects (acute toxicity, repeated-dose toxicity, genetic toxicity, and reproductive/developmental toxicity).

Pending submission of more complete information on the identity of the test substances and other important details in the robust summaries, EPA reserves judgement on the adequacy of these endpoints.

Acute Toxicity. There is a discrepancy between the LD_{50} values listed in the test plan and those in the robust summary. The acute oral LD_{50}s listed in Table 5 of the test plan are incorrect for CAS No. 793-24-8 (the listed value of >5000 mg/kg should be $\geq$2500 mg/kg) and CAS No. 3081-01-4 (the listed value of >2000 mg/kg should be 2100 mg/kg).

Ecological Effects (fish, invertebrates, and algae).

EPA reserves judgment on the adequacy of the submitted toxicity data on fish, daphnia, and green algae, pending adequate explanation on test conditions for these studies. The submitter needs to address deficiencies in the robust summaries to allow determination of data adequacy. Because these chemicals undergo rapid degradation (hydrolysis half-life 3 to 5 hours, photolysis (one example) half-life 2 hours), the test organisms will be exposed primarily to degradation products and the latter need to be properly identified and explained (see comments under Environmental Fate).

Specific Comments on Robust Summaries

General Comment

One set of robust summaries is labeled with CAS No. 3081-14-9 and chemical name *"p*-phenylene-diamine, N-1,4-dimethylpentyl-N'-phenyl"; the number and name do not match. The submitter needs to match all of the information in this data set to the appropriate chemical.

Physicochemical Properties

Water Solubility. The robust summary is inadequate for CAS No. 101-96-2. The value provided does not agree with model estimates or the water solubility values reported for N,N'-bis(1,4-dimethylpentyl)-p-phenylenediamine (a structurally similar compound, but with longer alkyl side chains). In addition, a quantitative value should be reported for CAS No. 15233-47-3. Finally, according to robust summaries submitted by the sponsor, the water solubility in Table 2 of the test plan appears to be incorrect for CAS No. 3081-01-4 (the listed units of g/L should be mg/L).

Environmental Fate

The submitter needs to provide clarification on the degradation products. For example, the submitter indicates in two cases that one of the hydrolysis products is "4-hydroxylamine," an obvious misnomer. A degradation product listed for CAS No. 3081-14-9 is not a possible degradation product of that substance but is consistent with the title chemical name (see General Comment above).

Health Effects

General Comments. Several robust summaries were missing important details, as discussed in the sections below. In addition, in the IUCLID data sets for CAS No. 68953-84-4, the submitter did not provide sections 1.1-1.4; however, many robust summaries for this chemical refer to these sections for the identity of the test substance. Furthermore, summaries for several chemicals provided only the commercial name of the products.

Acute Toxicity. Experimental details missing from some study summaries include sex, strain, group sizes, vehicle, test doses/concentrations, nature of the atmosphere in inhalation studies (gas, particulate, etc.), mortality-dose response, clinical signs, necropsy findings, and LD_{50}/LC_{50} confidence limits.

Repeated-Dose Toxicity. Experimental details missing from the study summaries include incidence data, magnitude of effects, and statistical significance of observed effects. Also, the NOEL and LOEL values appear to be transposed in the summary for CAS No. 3081-01-4. Finally, no NOAEL or LOAEL values were reported in the first summary for CAS No. 793-24-8.

Genetic Toxicity. The robust summary of the OECD Guideline 476 study for CAS No. 101-96-2 need to be clarified. The summary remarks refer to chromosomal aberrations, but the study type is described as a forward gene mutation assay. In addition, experimental details missing from some robust summaries include test concentrations, the use of positive and negative controls, number of replicates, and statistical analyses.

Reproductive Toxicity. Experimental details missing from the study summaries include incidence data, magnitude of effects, and statistical significance of observed effects. In the three generation study for CAS No. 793-24-8, it appears that the parental NOAEL should be 100 ppm (not 10 ppm).

Developmental Toxicity. In the study summaries, adverse effects are sometimes reported without incidence data, magnitude, or any indication of statistical significance. The first developmental study for CAS No. 68953-84-4 indicated a linear trend in decreasing fetal body weights with dose; it may be appropriate to derive a fetal toxicity NOAEL based on these effects. In the developmental toxicity study summary of CAS No. 101-72-4, 62.5 mg/kg/day is indicated as a developmental toxicity NOEL, but there was a statistically significant increase in incomplete ossification of more than one cranial bone at this dose. There was also a statistically-significant increase in incomplete ossification of more than one facial bone at 12.5 mg/kg/day. The submitter needs to address this apparent inconsistency regarding the developmental toxicity NOEL.

Ecological Effects

In general, the robust summaries did not provide enough detail. The submitter should consult EPA guidance documents for the preparation of robust summaries (http://www.epa.gov/opptintr/chemrtk/guidocs.htm). Commonly missing information included test substance purity, tested concentrations, number of organisms (or algal cultures) per concentration, solvent and negative control use and response, solvent concentration, complete mortality and/or response data, statistical methods used, test type, and water chemistry parameters. In addition, the submitter needs to provide accurate information on degradation products.

Followup Activity

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.

I U C L I D

D a t a S e t

Existing Chemical	ID: 68953-84-4
CAS No.	68953-84-4
EINECS Name	1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs.
EINECS No.	273-227-8

Producer Related Part	
Company:	ACC Rubber and Plastics Additives Panel
Creation date:	31-July-2000
Substance Related Part	
Company:	ACC Rubber and Plastics Additives Panel
Creation date:	31-July-2000

Printing date:	22-JAN-2003
Revision date:	
Date of last Update:	22-Jan-2003

Number of Pages:	51

Chapter (profile):	Chapter: 1.1, 1.2, 1.3, 1,4, 2.1, 2.2, 2.4, 2.5, 3.6.1, 3.1.1, 3.1.2, 3.3.1, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5,5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8, 5.9
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

Date: 22-Jan-2003

1. General Information ID: 68953-84-4

1.1 General Substance Information

Substance Type:
Physical Status: solid
Purity: 90 - 95 wt. %
Result: Molecular Weight: 274 (avg.)

1.1.1 Spectra

1.2 Synonyms

1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs.

Accinox 100

Blend of phenyl and tolyl p-phenylenediamines

DAPD

Mixed diaryl-p-phenylenediamines

Mixed di-aryl-p-phenylenediamines

Diaryl-p-phenylenediamines

Naugard 496

Vulkanox 3100

Wingstay 100

Polystay 100

WTR Number 4a

Nailax (Nailax B)

Remark: Complex reaction product containing;
N,N'-di(o-tolyl)-p-phenylenediamine;
N.N'-Diphenyl-p-phenylenediamine; and
N-Phenyl-N'-(o-tolyl)-p-phenylenediamine

Date: 22-Jan-2003

1. General Information ID: 68953-84-4

1.3 Impurities

CAS Number: 95-53-4
EINECS Number: 202-429-0
Chemical Name: o-Toluidine
Contents: < 0.1 wt %

CAS Number: 62-53-3
EINECS Number: 200-539-3
Chemical Name: aniline
Contents: < 0.1 wt %

CAS Number: 552-82-9
EINECS Number: 209-023-2
Chemical Name: Methyldiphenylamine
Contents: < 0.1 wt %

CAS Number: 122-39-4
EINECS Number: 204-539-4
Chemical Name: Diphenylamine
Contents: 1 - 5 wt %

1.4 Additives

2.1 Melting Point

Value:	90 - 105 degree C
Decomposition:	ambiguous
Method:	other: ASTM D-1519
Year:	1993
GLP:	no
Reliability:	(2) valid with restrictions Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.

31-JUL-2000 (35)

2.2 Boiling Point

2.3 Density

Type:	
Value:	
Method:	Other: ASTM D-891
Result:	Specific Gravity: 1.18
Reliability:	2) valid with restrictions Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.

31-Jul-2000 (34)

2.4 Vapour Pressure

2.5 Partition Coefficient

log Pow:	3.4 - 4.3
Method:	OECD Guide-line 117 "Partition Coefficient n-Octanol/Water), HPLC Method"
Year:	1995
GLP:	yes
Remark:	The product exhibits much lower values than DDT (6.2) which provides a benchmark for highly bioaccumulative chemicals. The test substance contains 3 major components.
Result:	# Methyl Groups -0 log Pow 3.37 # Methyl Groups -1 log Pow 3.82 # Methyl Groups -2 log Pow 4.28 The major components of the test substance displayed partition coefficients between 3.4 and 4.3.[as prescribed by 1.1-1.4 (Wingstay 100, mixed diaryl-p-phenylenediamines)]
Reliability:	(1) valid without restriction

01-AUG-2000 (29)

log Pow: > 3.7 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: for N,N'-Diphenyl-p-phenylenediamine
Reliability: (1) valid without restriction
20-FEB-2001 (9)

log Pow: > 4.3 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: For N-phenyl-N'-(o-tolyl)-p-phenylenediamine
Reliability: (1) valid without restriction
31-JUL-2000 (9)

log Pow: > 4.6 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: For N,N'-Di(o-tolyl)-p-phenylenediamine
Reliability: (1) valid without restriction
20-FEB-2001 (9)

3.1.1 Photodegradation

3.1.2 Stability in Water

Type:
Method:
Year: 1994 GLP: yes
Test substance: as prescribed by 1.1 - 1.4 (Mixed diaryl-p-phenylenediamines)
Remark: See Biodegradation Studies
Reliability: (1) valid without restriction
31-JUL-2000 (23)

3.3.1 Transport between Environmental Compartments

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, domestic
Concentration: 100 mg/l related to Test substance
Degradation: .64 % after 28 day
Result: other: not readily biodegradable
Method: OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year: 1994 GLP: yes
Test substance: as prescribed by 1.1 - 1.4 (Mixed diaryl-p-phenylenediamines)
Reliability: (1) valid without restriction
31-JUL-2000 (23)

Type: aerobic
Inoculum: activated sludge
Degradation: 0 % after 28 day
Method: other: OECD 301 Manometric Respirometry, modified according to EEC Round Robin Test "Assessment of Respirometry" DGX 1/283/82
Rev. 6, EEC Directive 79/831, Annex V, Part C
Year: 1990 GLP: yes
Test substance: as prescribed by 1.1 - 1.4 (Mixed diaryl-p-phenylenediamines)

Reliability: (1) valid without restriction
31-JUL-2000 (6)

3.6 BOD5, COD or BOD5/COD Ratio

Method: other: unknown
Method: other: unknown

Result: ThOD: 3056 mg/g
Reliability: (4) not assignable

(6)

Method: other: unknown
Method: other: unknown

Result: ThOD: 2.555 mg/mg
Reliability: (4) not assignable

(23)

3.7 Bioaccumulation

Species:	Cyprinus carpio (Fish, fresh water)
Exposure period:	56 day
Concentration:	.05 mg/l
BCF:	< 5000
Elimination:	
Method:	other: MITI Method for Testing the Degree of Accumulation of Chemical Substances in Fish Bodies
Year:	1998 GLP: yes
Test substance:	as prescribed by 1.1 - 1.4 (Wingstay 100, mixed diaryl-p-Phenylenediamines)
Method:	The test substance had an assumed purity of 100%. A pilot toxicity test used orange-red killifish (Oryzias latipes) (10 fish per level) exposed the test substance for 48-hours in a semi-static system. Stock solutions were prepared by dissolving the test substance and HCO-40 (hydrogenated castor oil; 20 times the amount of the test substance) in tetrahydrofuran. Following evaporation of the tetrahydrofuran,ion-exchanged water was added to the mixture to prepare a 500 mg/L stock solution of the test substance. Carp (Cyprinus carpio)was used as the test species for the Bioconcentration study. Based on the 48-hours toxicity results and analytical detection, the test concentrations used were Level 1 (high exposure level)-0.05 mg/L and Level 2 (low exposure level)-0.005 mg/L. The test tanks were 100 L glass tanks. The test solution was entered into mix tanks at a flow rate of two(2) mL/minute for the stock solution and 1600 mL/minute for the dilution water. For controls, HCO-40 was dissolved with ion-exchanged water to give a 800 mg/L solution. The duration of exposure was for 8-weeks. Dissolved oxygen in the test tanks was measured twice a week. The concentrations of the test substance in water for both Levels were analyzed twice per week throughout the study. The concentrations of the test substance in fish at both Levels were analyzed during Week -1, -2, -4, -6 and –8 {two (2) fish per week}. Control fish were analyzed at the initiation {two (2) fish} and at termination {two (2) fish} of exposure. Additional fish were subjected to analysis on Days -1, -5, and -8 following cessation of exposure on Study Day-56 to assess depuration of test substance from fish tissues. All tissue and test water samples were analyzed using high performance liquid chromatography (HPLC).

Water levels were analyzed by loading large volumes on C18 Sep Pak mini-column, which was then eluted from column with Acetonitrile containing 0.1% Formic acid. The final volume of eluate was 5 mL. Test fish were analyzed by measuring weights, body lengths, chopping into pieces, and extracting with Acetonitrile. The mixture was centrifuged {7000xg. Five (5) minutes} and the supernatant was filtered with absorbent cotton to a volume of 100 mL. Two (2) separate samples were analyzed to assess Diphenylamine (DPA) and Diaryl p-phenylenediamine (diaryl-PPD) components (87% of complex)and to assess higher molecular weight components (13 % of complex). All recovery and blank tests were carried out in duplicate.

Remark: For DPA and DPPD compounds, methyl substitution increased bioaccumulation in carp, consistent with increasing log Po values. Substantial variation occurred at each time point due to use of data from a maximum of 2 fish. While this project provided substantial data, further work was needed to calculate BCFs according to western (OECD) concepts, and to apply appropriate statistics to these data so as to provide basis for interpretation.

To address this issue, a project was conducted by McLaren Hart entitled "Statistical Calculations of Data from a Bioaccumulation Study with WINGSTAY 100 in Carp", November 25,1998. The analysis employed Monte Carlo methods; the maximum BCF value (Pk 5) was 6600, and depuration data confirmed the attainment of tissue steady state levels of WINGSTAY 100 components within 3 weeks. Depuration was confirmed to be < 5 days for all components.
Orange-red killifish (Oryzias latipes) were used in the pilot toxicity test.

Result: Bioconcentration Test: The laboratory had difficulty maintaining nominal concentrations, possibly due to rapid uptake and metabolism by the fish and partioning to tank surfaces. The test concentrations ranged from 60 to 100% of the nominal values. The Bioconcentration Factors (BCFs) were calculated from individual data for fish at each time point and by using time-weighted averages for water concentrations. Since the test substance was a complex reaction product with numerous peaks, there was a high degree of variability in the fish data resulting in a large range of BCF values (20-221 for Peak 1; 128-659 for Peak 2; 269-2460 for Peak 3; 776-3640 for Peak 4; 2980-11300 for Peak 5). Depuration results for components indicated half-lives were below five (5) days for all components with the exception to one (1) estimate of 44-days for Peak 5. This inconsistent value appears to be suspect since it is much higher than the value of 4.7 days that was obtained for the same Peak in the other concentration. Also, the value is inconsistent with the trend Observed for half-lives for Peaks 1 through 4.

Bioconcentration Factors (BCFs) were calculated by using individual data points, including those prior to reaching steady-state. Estimates of steady-state through the use of Monte Carlo modeling improved the estimations of the BCFs. The bioaccumulation data and depuration data can be used together in performing analyses, particularity when the collected bioaccumulation data contained information on half-lifes(i.e., time to reach steady-state). The Monte Carlo "best estimates" for BCFs were < 5000 for all components except Peak 5 which had a BCF of approximately 7000.
Pilot Toxicity Test: The 48-hour LC50 result for the test substance in orange-red killifish was 17.2 mg/L. **Please note**: this concentration was achieved only through the use of a surfactant {Hydrogenated Castor Oil (HCO-40)}, and is far above the test substance solubility in water (approximately 2 mg/L). MITI guidelines recommend levels for Bioaccumulation testing to be at 1/1000 and 1/10,000 of the LC50 value. The lower value would have been below the quantitation range; thus, 0.005 and 0.05 mg/L were chosen.

Test condition: Two (2) test concentrations were used: Level 1 (high exposure level)-0.05 mg/L and Level 2 (low exposure level)-0.005 mg/L

Reliability: (1) valid without restriction

(10)

Date: 22-Jan-2003
4. Ecotoxicity ID: 68953-84-4

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type:	flow through
Species:	Cyprinus carpio (Fish, fresh water)
Exposure period:	14 day
Unit:	mg/l Analytical monitoring: yes
NOEC:	.28
LC50:	.43
Method:	OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"
Year:	1996 GLP: yes
Test substance:	Wingstay 100 (mixed di-aryl-p-phenylenediamines)
Method:	Test water was generated by adding the test substance in acetone to a larger volume of water which was stirred, allowed to settle, and then siphoned to a stock solution holding tank. This stock solution was then metered into exposure tanks for the fish experiments. A range-finding trial exposed carp to nominal levels of 2.5, 5, 10, and 25 mg/L (ppm) of the test substance. Survival rates were up to 80% within the first 48 hours for the three (3) highest dose levels and the 2.5 mg/L induced no mortality in the first 48 hours although 90% deaths were seen through Day six (6). In the definitive phase, duplicate test tanks contained 10 carp each and the test substance nominal concentrations of 0, 0.1, 0.23, 0.51, 1.1, and 2.5 mg/L (ppm). Chemical analysis (HPLC) of the test substance in the test tanks on Days -0, -3, -7, and -14 showed that mean concentrations for the 14-day test period were 0.053, 0.12, 0.19, 0.28, and 0.67 mg/L (ppm). Fish densities were 0.35 g biomass/L flowing test solution per day. Tank volume turnover for the flow-through system was 6.5/day. Carp were monitored daily for mortality and signs of erratic swimming behavior for 14 days during exposure. Body weights and lengths were recorded for representative fish prior to study initiation, and on all test fish on Day 14. A LC50 value was then calculated.
Result:	Carp died only at the highest test substance concentration; 2/20 on Day-3, 7/20 on Day-7, and 20/20 by Day-14. Other findings at the 0.67 mg/L (ppm) level included darkened pigmentation on the fish (likely due to adsorption of the test chemical), lethargic swimming behavior, and loss of equilibrium. There were no test substance-related effects on body lengths or weights.
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (1) valid without restriction
20-FEB-2001 (30)

Date: 22-Jan-2003
4. Ecotoxicity ID: 68953-84-4

Type: flow through
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 14 day
Unit: mg/l Analytical monitoring: yes
NOEC: .14
LC50: .26
Method: OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"
Year: 1997 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Test water was generated by adding the test substance in acetone to a larger volume of water which was stirred, allowed to settle, and then siphoned to a stock solution holding tank. This stock solution was then metered into exposure tanks for fish experiments. A preliminary study in trout was performed using nominal concentrations of the test substance of 0.1, 0.23, 0.51, 1.1, and 2.5 mg/L. Mortality rates were 100% at the highest level by Day-3, and was 80% by Day-7 at 1.1 mg/L.

In the definitive phase, duplicate test tanks contained 10 trout each, Test substance nominal concentrations of 0, 0.094, 0.19, 0.38, 0.75, and 1.5 mg/L (ppm) were chosen. Chemical analysis (HPLC) of the test substance in the test tanks on Days -0, -7 and -14 showed that mean concentrations for the 14-day test period were 0.062, 0.093, 0.14, 0.35, and 0.66 mg/L (ppm). Fish densities were 0.079 g biomass/L flowing test solution per day. Tank volume turnover for the flow-through system was 6.5/day. Fish were monitored daily for mortality and signs of erratic swimming behavior for 14-days during exposure. Body weights and lengths were recorded for representative fish prior to study initiation, and on all test fish on Day-14. LC50 values were calculated for 96-hours and 14-days.

Result: Fish died only at 0.35 and 0.66 mg/L concentrations; 0/20 and 1/20 died by Day-2 and 1/20 and 19/20 by Day -4 , respectively. Further, 100 % of the high dose (0.66 mg/L) fish died by Day-5 and 17/20 of the 0.37 mg/L fish by Day-14. Other findings at the two highest levels included darkened pigmentation of the fish, lethargic swimming behavior, and loss of equilibrium. There were test substance-related effects on 14-day body lengths and weights in the 0.35 mg/L group. The calculated LC50 for the test substance in the study at 96-hours was 0.48 mg/L and 0.26 mg/L at 14-days. The No Observed Effect Concentration (NOEC) was 0.14 mg/L at 96-hours and 14-days.

Reliability: (1) valid without restriction
31-JUL-2000

(38)

4.2 Acute Toxicity to Aquatic Invertebrates

Species:	Daphnia magna (Crustacea)
Exposure period:	48 hour(s)
Unit:	mg/l Analytical monitoring: yes
NOEC:	.36
EC50:	1.8
Method:	OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year:	1996 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: A range-finding study used ten (10) 24-hour old daphnids exposed to nominal levels of 0, 13,22,36,60, and 100 mg/L of the test substance. Immobilization (15%) of the daphnids occurred at the highest level (100 mg/L). Sublethal lethargy was observed at all but the lowest test concentration (13 mg/L). Brown matter, apparently the test substance since brown precipitate was observed in the media, was observed to adhere to both surviving and non-surviving daphnids.

In the definitive phase, duplicate aquaria containing 10 daphnids each and test substance nominal concentrations of 0, 1.3, 2.2, 3.6, 6.0 and 10 mg/L (ppm) were prepared. Mean values for the test substance concentrations in the test media were determined by averaging chemical analyses (HLPC) of 0-hours and 48-hours.

Daphnia immobilization and aquaria observations were made at 24- and 48-hours following the study initiation. From these data, an Effective Concentration in one-half the organisims (EC50) and a No Observed Effect Concentration (NOEC) were estimated.

Result: Measured concentrations of the test substance ranged from 19 to 29% of nominal levels. At the highest concentration (1.8 mg/L), 25 % of the daphnids were immobilized at 48-hours of exposure. For the 0.68 and 1.1 mg/L groups, Five (5) % of the daphnids were immobile. No immobilization was observed at 0.20 and 0.36 mg/L exposures. Lethargic activity was not observed at any treatment level. Brown particulates, perhaps the test substance, were observed to adhere to the test daphnids, with some buoyed to the surface of the aquaria by this particulate material. The results indicated that the EC50 for the test substance was 1.8 mg/L. The No Observed Effect Concentration (NOEC) was shown to be 0.36 mg/L.

Reliability: (1) valid without restriction
31-JUL-2000 (28)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species:	Selenastrum capricornutum (Algae)
Endpoint:	biomass
Exposure period:	72 hour(s)
Unit:	µg/l Analytical monitoring: yes
NOEC:	4.3
EC10:	4.3
EC50:	18
Method:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year:	1996 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: A range-finding trial used nominal levels of 0, 1,10, 100, and 1000 ug/L (ppb) of the test substance and a solvent control in algae cultures (approximately 1x104 cells per flask). Following 72-hours incubation, algal cell densities were determined using a hemacytometer. Values were 127,76,109,69 and 1%, respectively, of the solvent control response. These values were used to set exposures for the definitive phase.

In the definitive phase, triplicate algal cultures were exposed to the test substance at nominal concentrations of 16, 31, 63,130, 250, and 500 ug/L (ppb). Cell densities were monitored at 24-, 48-, and 72-hours following study initiation. From these data, EC50 (50% decrease) values for Biomass (EbC50) and Growth Rate (ErC50) were calculated. Test substance concentrations in the test media were determined at 0- and 72-hours using HLPC. The mean concentrations were 7.5, 13, 14, 28, 50, and 79 ug/L (ppb).

Result: The inhibitions of algae Growth Rates for the test substance in the definitive 72-hour study were 0, 2, 15, 20, 32, and 38% (relative to pooled control values) for the measured test substance concentrations of 7.5, 13, 14, 28, 50, and 79 ug/L (ppb). Corresponding inhibitions of Biomass generation were 15, 41, 59, 63, 81, and 91%. Individual cell appearances were found microscopically to be normal for surviving cells except cellular bloating was noted at the highest exposure level. Calculations indicated that the ErC50 for the test substance was > 79 ug/L (ppb) while the EbC50 was 18 ug/L (ppb). The No Observed Effect Concentrations (NOECs) were assumed to be equivalent to EC10 values, and accordingly were EbC10 = 4.3 ug/L (ppb) and ErC10= 31 ug/L (ppb).

The EC50 values for the test substance ranged from 18 to > 79 ug/l (ppb) for Biomass increases and Growth Rates. The NOECs ranged from 4.3 to 31 ug/L (ppb) for these parameters.

Reliability: (1) valid without restriction
31-JUL-2000 (31)

Date: 22-Jan-2003
4. Ecotoxicity ID: 68953-84-4

Species:	Selenastrum capricornutum (Algae)
Endpoint:	growth rate
Exposure period:	72 hour(s)
Unit:	µg/l Analytical monitoring: yes
NOEC:	31
EC10:	31
EC50:	> 79
Method:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year:	1996 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: A range-finding trial used nominal levels of 0, 1,10, 100, and 1000 ug/L (ppb) of the test substance and a solvent control in algae cultures (approximately 1x104 cells per flask). Following 72-hours incubation, algal cell densities were determined using a hemacytometer. Values were 127,76,109,69 and 1%, respectively, of the solvent control response. These values were used to set exposures for the definitive phase.

In the definitive phase, triplicate algal cultures were exposed to the test substance at nominal concentrations of 16, 31, 63,130, 250, and 500 ug/L (ppb). Cell densities were monitored at 24-, 48-, and 72-hours following study initiation. From these data, EC50 (50% decrease) values for Biomass (EbC50) and Growth Rate (ErC50) were calculated. Test substance concentrations in the test media were determined at 0- and 72-hours using HLPC. The mean concentrations were 7.5, 13, 14, 28, 50, and 79 ug/L (ppb).

Result: The inhibitions of algae Growth Rates for the test substance in the definitive 72-hour study were 0, 2, 15, 20, 32, and 38% (relative to pooled control values) for the measured test substance concentrations of 7.5, 13, 14, 28, 50, and 79 ug/L (ppb). Corresponding inhibitions of Biomass generation were 15, 41, 59, 63, 81, and 91%. Individual cell appearances were found microscopically to be normal for surviving cells except cellular bloating was noted at the highest exposure level. Calculations indicated that the ErC50 for the test substance was > 79 ug/L (ppb) while the EbC50 was 18 ug/L (ppb). The No Observed Effect Concentrations (NOECs) were assumed to be equivalent to EC10 values, and accordingly were EbC10 = 4.3 ug/L (ppb) and ErC10= 31 ug/L (ppb).

The EC50 values for the test substance ranged from 18 to > 79 ug/l (ppb) for Biomass increases and Growth Rates. The NOECs ranged from 4.3 to 31 ug/L (ppb) for these parameters.

Reliability: (1) valid without restriction
31-JUL-2000 (31)

Date: 22-Jan-2003
4. Ecotoxicity ID: 68953-84-4

4.4 Toxicity to Microorganisms e.g. Bacteria

Type:	aquatic
Species:	activated sludge
Exposure period:	30 minute(s)
Unit:	mg/l Analytical monitoring: no
EC50:	> 10000
Method:	ISO 8192 "Test for inhibition of oxygen consumption by activated sludge"
Year:	1993 **GLP:** yes
Test substance:	as prescribed by 1.1 - 1.4
Reliability:	(1) valid without restriction

(6)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type:	LD50
Species:	rat
Strain:	
Sex:	no data
Number of Animals:	
Vehicle:	
Value:	> 2000 mg/kg bw
Method:	other: Directive 84/49/EEC, B.1
Year:	1990 GLP: yes
Test substance:	as prescribed by 1.1 - 1.4
Reliability:	(1) valid without restriction

01-AUG-2000 (7)

Type:	LD50
Species:	rat
Strain:	
Sex:	male/female
Number of Animals:	10
Vehicle:	other: corn oil
Value:	> 5000 mg/kg bw
Method:	other: US EPA 40CFR798.2650, Oral Toxicity-Limit Test
Year:	1993 GLP: yes
Test substance:	as prescribed by 1.1 - 1.4

Method: Five (5) male and five (5) female young adult rats (Sprague-Dawley) were administered a single dose of the test substance by gavage. The test substance was dispersed in corn oil (Sigma Chemical Company) and administered at a dosage of 5000 mg/kg. The animals were observed for clinical signs of toxicity at approximately 1-, 4- and 24-hours following administrations on the day of dosing and daily thereafter for 14-days. Body weights were recorded on Day-0, Day-7 and Day-14. All animals were subjected to a gross necropsy at study termination.

Result: One (1) animal died during the 14-day observation period. Clinical signs observed included decreased activity, decreased muscle tone, and diarrhea. No significant impairment on body weight gains were noted in either the male or female rats. Necropsy of the animal that died during the study revealed discolored kidneys, spleen, and liver. No visible lesions were observed in any of the animals at terminal necropsy. The estimated acute oral LD50 (combined sexes) for the test substance was determined to be > 5000 mg/kg.

Reliability: (1) valid without restriction

01-AUG-2000 (20)

Date: 22-Jan-2003
5. Toxicity ID: 68953-84-4

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: > 4000 mg/kg bw
Method: other
Year: 1959 GLP: no
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Result: No animals died at the single high dose of 4000 mg/kg.

Reliability: (4) not assignable
01-AUG-2000 (39)

5.1.2 Acute Inhalation Toxicity

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain:
Sex: male/female
Number of
Animals: 10
Vehicle: other
Value: > 2000 mg/kg bw
Method: OECD Guide-line 402 "Acute dermal Toxicity"
Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Albino rabbits (five males and five females) were shaved in the caudal portion of the animals' trunks. One (1) day later, a 2000 mg/kg dose of 40 mesh test substance (obtained by grinding in mortar/pestle) was placed onto the skin sites (approximately 10% of the body surface areas). The application sites were then covered with gauze, plastic, and elastic wraps and finally secured with non-irritating tape. After 24-hours of skin contact to the exposure areas, the gauze patches were removed and adhering test substance removed with moistened gauze. Skin test sites were scored for signs of erythema (redness) and edema (swelling) according to Draize procedures from Day-1 to Day-14 following cessation of exposures. Animals were observed for adverse clinical signs, mortality, and body weights (Day-0, Day-7, and Day-14). Necropsies were performed on the final day of observations (Day-14).

Remark: A limit test

Result: The test substance induced no deaths or apparent adverse clinical signs. Mild irritation (Grades 1,2 erythema; Grade 1 edema) was seen at skin sites of treated rabbits for periods ranging from Day-1 to Day-10. Staining of skin was noted due to the dark color of the test substance. A body weight decrease was seen in one (1) of the ten (10) rabbits between Day-7 and Day-14. No compound-related non-dermal findings were observed in the study. No mortality or adverse clinical/necropsy changes were observed associated with the test substance. The dermal LD50 for the test substance was shown to be > 2000 mg/kg.

Reliability: (1) valid without restriction
01-AUG-2000 (27)

5.2.1 Skin Irritation

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classification: not irritating
Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1991 GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Exposure period: 4 hours
Reliability: (2) valid with restrictions
(8)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classification: not irritating
Method: other: A 20% suspension of the material was applied to the shaved test site of six albino rabbits.
Year: 1959 GLP: no
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)
Reliability: (4) not assignable
(39)

Date: 22-Jan-2003
5. Toxicity ID: 68953-84-4

Species: rabbit
Concentration: undiluted

Exposure: Occlusive
Exposure Time: 4 hour(s)

Number of Animals: 6
PDII: .46
Result: slightly irritating
EC classification: not irritating
Method: Draize Test
Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Albino rabbits (six females) were shaved in the caudal Portion of the animals' trunks. One (1) day later, 0.5 grams of 40 mesh test substance (obtained by grinding in mortar/pestle) was placed on a one (1) inch squares of cotton gauze, moistened with water, applied to the skin sites, and secured with non-irritating tape. After 4-hours of skin contact exposures, the gauze patches were removed and adhering test substance removed with moistened gauze. Skin test sites were scored for signs of erythema (redness) and edema (swelling) according to Draize procedures at 1-, 24-, 48-, and 72-hours following cessation of exposures. Gross necropsies were performed on the animals following final scoring of the skin sites.

Result: The test substance induced no deaths or apparent adverse clinical or postmortem signs. Slight erythema (redness) was seen at skin sites of five (5) out of six (6) treated rabbits for maximum periods ranging from 1- to 48-hours. Staining of skin was noted due to the dark color of the test substance. The calculated irritation score was 0.46. The test results indicate an irritation rating as a "SLIGHT IRRITANT" and as a "NON-CORROSIVE".

Reliability: (1) valid without restriction

(26)

5.2.2 Eye Irritation

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: not irritating
EC classification: not irritating
Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1991 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Remark: Exposure period: 24 hours

Reliability: 2) valid with restrictions
(8)

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: 72 hour(s)
Comment: rinsed after (see exposure time)
Number of Animals: 9
Result: slightly irritating
EC classification: irritating
Method: Draize Test
Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: The eyes of albino rabbits (9-both genders) were examined using fluorescein dye and UV light for evidence of corneal damage and dye retention. Animals found to be acceptable received approximately 0.06 grams (0.1 mL) of 40 mesh test substance (obtained by grinding in mortar/pestle) applications to the right eyes. After 30-seconds of eye contact to the test substance, a water rinse was applied to three (3) of the nine (9) rabbits in an attempt to minimize chemical irritation. Left eyes were untreated and served as control sites. Eyes were assessed for signs of gross corneal, iridal, or conjunctival injury according to Draize procedures at 1-, 24-, 48-, and 72-hours (7-days for one (1) rabbit with eye damage at 72-hours). Fluorescein dye exams were conducted at 24-hours.

Result: The test substance induced no adverse clinical signs. No corneal damage was induced in any of the unrinsed rabbits although one (1) out of six (6) rabbits exhibited dye retention judged to be non-chemically related. Conjunctival {six (6) of six (6) and iridal (one (1) of six (6)}changes were seen in unrinsed rabbits primarily at the 1-hour inspection. All adverse findings were resolved by 72-hours except for one (1)rabbit with conjunctival redness which resolved by 7-days.The rinsed group exhibited some conjunctival irritation up to 72-hours. Irritation mean scores for unrinsed rabbits ranged from 8.2 (1-hour) to 0.33 (72-hours) to 0.0 (7-Days). Rinsed rabbits scores were 5.3 (1-hour) to 0.0 (72-hours). The test substance produced a mild irritation in rabbit eyes which was shown to be reversible. The test substance is considered to be a "MILD IRRITANT" to the eye.

Reliability: (1) valid without restriction

(25)

5.3 Sensitization

Type:	Guinea pig maximization test			
Species:	guinea pig			
Concentration:	Induction	5	% active substance	intracutaneous
	Induction	100	% active substance	intracutaneous
	Challenge	25	% active substance	occlusive epicutaneous
Number of Animals:	36			
Vehicle:				
Result:	sensitizing			
Classification:	sensitizing			
Method:	OECD Guide-line 406 "Skin Sensitization"			
Year:	1995		GLP: yes	
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)			

Method: Two (2) range finding trials (topical and intradermal injection) in two (2) male and two (2) female shaved albino guinea pigs were run which showed that the test substance at concentrations of 100% and 5% were appropriate for the definitive study, respectively. In the induction phase of the test, twenty test animals were given pairs of intradermal (0.1 mL) injections of 1) Freund's adjuvant, 2) %5 test substance in 0.5% acetone in propylene glycol, and 3) test substance + Freund's adjuvant at opposite sites from the animals' dorsal midline on Day-0. Appropriate negative and positive {2,4-Dinitro-1-chlorobenzene(DNCB)}controls were run on other animals. Topical induction exposures (48-hours) with site occlusion were done 7-days later following 24-hours test site exposure to Sodium lauryl sulfate.

Challenge (dermal)exposures were performed on Day-21 with both 25% (in acetone/mineral oil) and 100% test substance for 24-hours. Test animals were graded for dermal signs on the first and 2nd days following the challenge dosing. A dermal rechallenge trial was conducted on Day-28 by applying the test substance(25 and 100%) to these same animals. Dermal examinations were again performed one (1) and two (2) days later.

Result: The test substance induced no adverse clinical signs. Weak skin responses (erythema and edema) were observed in 25% test substance-treated challenge controls and in test substance-induced animals. Mean scores were not significantly different from the controls although a greater number of induced animals exhibited "slight but confluent or moderate patchy erythema". The test substance at 100% produced the same results. However, upon rechallenge of these animals 7-days later with 25 and 100% test substance, severities of dermal responses increased in test substance induced animals as did the mean dermal scores (0.8-1.0) relative to challenge (non-induced) controls (0.0-0.3). The positive control agent (DNCB) produced dermal scores at 24- and 48-hours of 0.3 and 0.5 for previously untreated animals versus scores of 2,5 for DNCB-induced guinea pigs. The test substance is considered to be a contact sensitizer.

Reliability: (1) valid without restriction

(24)

5.4 Repeated Dose Toxicity

Species:	rat	Sex: male/female
Strain:	Fischer 344	
Route of admin.:	oral feed	
Exposure period:	28 days	
Frequency of treatment:	Daily	
Post. obs. period:	2 weeks	
Doses:	0, 7.5, 30 and 120 mg/kg/day	
Control Group:	yes, concurrent vehicle	
NOAEL:	7.5 mg/kg	
LOAEL:	30 mg/kg	
Method:	other: Oral 4-week dietary study	
Year:	1996	GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)	

Method: The test substance was prepared by grinding in a coffee mill, sieved through a 125 um mesh screen and mixed with rodent diet NIH-07 at 0, 120, 470, 1900 ppm (0, 7.5, 30, and 120 mg/kg/day). Stability, homogeneity, and dose verification were performed to confirm compliance with protocol. The prepared dosed feed was presented to 14 male and 14 female rats (Fischer 344) per test group at twelve weeks of age for four (4) weeks. Six (6) rats/sex/group were held for post-exposure in two (2) week recovery groups. Test rats were monitored for body weights, feed consumption, and clinical signs. Collections were performed on six (6) or three (3) rats/sex/group at 28-days and 42-days sacrifice periods for blood (hematologies and clinical chemistries) and urinalyses, respectively. Necropsies were performed on all rats, and organs were weighed (liver, kidneys, pituitary, uteri, heart, brain, spleen, thyroids, adrenals, testes, and ovaries). These and other major organs were preserved in formalin, stained with H&E, and subjected to microscopic evaluations. Liver, kidney, and urinary bladder slices were subjected to immunohistochemical staining for proliferating cell nuclear antigen (PCNA) for assessment of cellular division.

Result: The test substance was shown to be completely stable in diets for 46-days. Mixing procedures produced homogeneous diets that were found within 10% of target concentrations. No compound-related deaths occurred. The body weights were not affected in male rats whereas the high dose female rats displayed 5% body weight decreases during study weeks two (2) through four (4). Food consumption was decreased in the high dose males and in the mid- and high dose females mainly during study weeks two (2) through four (4).

Various test substance-induced hematological changes occurred that included: increased mean corpuscular volumes and decreased mean corpuscular hemoglobin concentrations (high dose males and females) and blood bilirubin and cholesterol increases (high dose males and females). Most blood endpoints tended to approach control levels during week two (2) of the recovery period. No dose-related urinary changes were seen. Organ weight increases were seen at 28-days for liver and kidneys(high dose males and females; mid-dose females) and heart and spleen (high dose females). Only the kidney weights did not reach control levels by 42-days. There were no gross tissue or microscopic changes related to the test substance. Proliferating cell nuclear antigen (PCNA) exams showed cell division changes for: increases for liver cells (High dose males and females and mid-dose males at 28-days only); changes for kidney cells (decreases in high dose females at 28-days and increases in high dose males and females at 42-days; and increasing trend in urothelial cells in bladder (low and mid-dose males and females at 28-days). Macrocytic anemia was the primary change in rats related to the test substance administration. This change was reversible within 2 weeks following dietary exposure as were liver weight and serum cholesterol elevations. These changes were very minor, and had no apparent toxicological significance in this study. The lack of dose-responsiveness in the PCNA data provides results of uncertain importance to the assessment of the toxicity of this test substance.

Reliability: (1) valid without restriction
02-AUG-2000

(11)

Species:	rat Sex: male/female
Strain:	other: Fischer 344/N TacfBR
Route of admin.:	gavage
Exposure period:	21 days
Frequency of treatment:	Daily
Post. obs. period:	
Doses:	0, 0.1, 0.3, 1.0, and 3.0 g/kg/bw
Control Group:	yes, concurrent vehicle
LOAEL:	100 mg/kg bw
Method:	other: Oral 3-Week Range-Finding Study
Year:	1994 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Remark: A 4-week diet-study was also conducted.

Result: Doses of 1.0 and 3.0 g/kg/day of WINGSTAY 100 (mixed diaryl-p-phenylenediamines) were administered by gavage for up to 6 days were lethal for male and female F344 rats. The only pertinent gross finding of all unscheduled deaths was the paleness of most external surfaces and viscera. The mid-low (0.3 g/kg/day) and low(0.1 g/kg/day) doses caused time and dose related significant body weight loss, liver weight increase and hepatocellular labeling index increase at 0.1 g/kg. Therefore, in the subchronic studies, the recommended daily dose of WINGSTAY 100 (mixed diaryl-p-phenylenediamines) should not exceed 100 mg/kg/day, if administered by gavage.

Test substance Preparation: The test substance was prepared in an olive oil suspension for dosing.

Reliability: (1) valid without restriction
02-AUG-2000

(5)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test

System of testing: Ames/E. coli preincubation; Salmonella typhimurium TA-98, 100, 1535, 1537, 1538, and WP2 uvrA

Concentration: Salmonella stains without S9 activation: 0.167, 0.5, 1.67, 5, 16.7, and 50 ug/plate; Salmonella strains with S9 activation: 1.67, 5, 16.7, 50, 167, and 500 ug/plate; E.coli with/without S9 activation: 1.67, 5, 16.7, 50, 167, and 500 ug/plate

Metabolic activation: With and without

Method: other: Japan's Industrial Safety & Health Law, a combination of OECD Guidelines 471 and 472.

Result: Positive. The test substance was shown to cause mutations in Ames/Salmonella strains TA1538 and TA98 with S9 activation.

In a preliminary assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA1537, TA98, TA100, and WP2 uvrA with S9 metabolic activation, and in tester strains TA1535, TA1538, TA98, TA100 and WP2 uvrA without S9 activation, approximated the concurrent negative controls. However, statistically significant, increases in reverent frequencies, to approximately 1.7- to 2.5-fold control values, were observed in tester strain TA1538 with S9 metabolic activation and in tester strain TA1537 without S9 metabolic activation. In addition, the increases observed in strain TA1538 with S9 metabolic activation were dose dependent.

In a confirmatory assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA100, and WP2 uvrA with metabolic activation, and in tester strains TA1535, TA1538, TA98, TA100, and WP2 uvrA without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1537, TA1538, and TA98 with metabolic activation. Statistically significant increases in reverent frequencies, to control values, also were observed in tester strain TA98 without S9 metabolic activation. However, these latter increases apparently were not dose related.

The test substance was re-evaluated in all five Salmonella strains with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation. Reverent frequencies for all doses of the test substance in tester strains TA1535, TA1537, and TA100 with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation, approximated or were less than control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1538 and TA98 with S9 metabolic activation. All positive and negative control values in all assays were within acceptable limits.

Year: 1993 GLP: yes

Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
04-AUG-2000

(16)

Type: Ames test

System of testing: Ames/Salmonella-E.coli Liquid Pre-incubation Assay in Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 And in E.coli strain WP2 uvrA.

Concentration: Salmonella strains with S9: 1.67, 5, 16.7, 50, 167, and 500 ug/plate; Salmonella strains without S9: 0.167, 0.5, 1.67, 5, 16.7, and 50 ug/plate; E.coli with/without S9: 1.67, 5, 16.7, 50, 167, and 500 ug/ plate.

Metabolic activation: With and without

Method: other: Japan's Industrial Safety & Health Law, a combination of OECD Guidelines 471 and 472.

Result: Positive. The test substance was shown to cause mutations in Ames/Salmonella strains TA1537, TA1538 and TA98 with S9 metabolic activation.

In a preliminary assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA1537, TA100, and WP2 uvrA with and without S9 metabolic activation approximated the concurrent negative controls. However, statistically significant, increases in reverent frequencies, to control values, were observed in tester strains TA1538 and TA98 with S9 metabolic activation. In addition, the increases observed in strain TA1538 with S9 metabolic activation were dose dependent.

In a confirmatory assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA100, and WP2 uvrA with metabolic activation, and in tester strains TA1535, TA1537, TA1538, TA100, and WP2 uvrA without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1537, TA1538, and TA98 with metabolic activation. Statistically significant increases in reverent frequencies, to control values, also were observed in tester strain TA98 without S9 metabolic activation. However, these latter increases apparently were not dose related.

The test substance was re-evaluated in all five Salmonella strains with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation. Reverent frequencies for all doses of the test substance in tester strains TA1535, and TA100 with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1537, TA1538, and TA98 with S9 metabolic activation. All positive and negative control values in all assays were within acceptable limits.

Year: 1994 GLP: yes

Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
04-AUG-2000

(17)

Date: 22-Jan-2003
5. Toxicity ID: 68953-84-4

Type:	Cytogenetic assay
System of testing:	Chromosomal aberration assay in CHO cells
Concentration:	0.4, 2, 4, and 25 ug/mL
Metabolic activation:	With and without
Result:	Negative. The test substance was judged negative (non-clastogenic)based on its inability to reproducibly induce dose-related increases in structural chromosomal aberrations in CHO cells. Analysis of the data for the 24-hour treatment with the test substance indicated that there were statistically significant dose-related increases in the frequency of aberrations/cell and proportion of aberrant metaphases at doses 2 and 4 ug/mL. The data for the 2 and 4 ug/mL doses produced a statistically significant linear trend when analyzed by the Cochran/Armitage Linear Trend Test. To verify the biological significance of this finding, the 24-hour treatment was repeated. In the confirmatory assay, the test substance was re-evaluated at doses of 25 ug/mL with S9 metabolic activation (5-hour treatment) and 0.4, 2, and 4 ug/mL without S9 metabolic activation (24-hour treatment). Analysis of the data for the 5-hour treatment did not produce statistically significant increases in aberrations/cell or in proportion of aberrant metaphases. Analysis of the data for the 24-hour treatment indicated a statistically significant increase in aberrations/metaphase at the mid-dose (2 ug/mL) with S9 metabolic activation but there were no significant increases in the proportion of aberrant metaphases. However, when the data for 2 ug/mL (0.045 + or - 0.208) were compared to the untreated control data (0.025 + or - 0.157) or to Pharmakon historical acetone data (0.034 + or - 0.021), there were no statistically significant increases in the frequency of aberrations/metaphase. Therefore, the positive finding in the t-test for 2 ug/mL was considered a statistical artifact with no biological significance. There were no other statistically significant increases in aberration/metaphase or in the proportion of aberrant metaphases at any of the remaining dose levels for the 24-hour treatment.
Method:	OECD Guide-line 473 "Genetic Toxicology: In vitro Mammalian Cytogenetic Test"

In the structural Chromosomal Aberration assay, duplicate cultures were established for each dose level. Three treatment schedules were used: a) First set of cultures were treated for 5-hours with the appropriate dose of the test sample in Ham's F12 serum free (F12SF) medium either in the presence or absence of S9 metabolic activation along with concurrent negative and positive controls followed by three (3) Puck's saline washes and medium replacement; b) Second set of cultures were treated for 24-hours with the test substance or control articles in Ham's F12 medium containing five (5) % serum (F12FCM5%) without S9 metabolic activation, and; c) Third set of cultures were treated for 48-hours with the test substance or control articles in F12FCM5% medium without S9 metabolic activation. Two (2) to three (3) hours prior to harvest, Colcemid (2X10-7M) was added to all sets of cell cultures to arrest dividing cells in metaphase. CHO cells were harvested at the appropriate time and metaphase slides were prepared and stained.

The data from one hundred metaphases from each culture (200 metaphases per dose point) were pooled for statistical analysis. Data were evaluated by using the chi-square of aberrant versus normal cells while comparing each dose level to its concurrent negative control. The data were also analyzed for statistical significance by pairwise t-tests comparing the number of aberrations per cell in each treated dose versus the negative control.

Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
20-FEB-2001

(19)

Type: DNA damage and repair assay
System of testing: E. coli Pol A1- Liquid Suspension Assay

Concentration:

Metabolic activation: Without

Result: Positive
Method: Other
Year: 1980 GLP: no

Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000

(33)

Type: other: Transformation Assay
System of testing: Balb/3T3 In Vitro Transformation Assay

Concentration: .01 ug/ml to 1.0 ug/ml

Metabolic activation: Without

Result: Negative
Method: other
Year: 1981 GLP: no

Test substance: Nailax (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.
04-AUG-2000

(12)

Type: other: Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes
System of testing: Hepatocytes form male Fischer 344 (F344/Crl) rats

Concentration: Slightly above their limits of solubility

Metabolic activation: Without

Result: Negative. In all the Unscheduled DNA Synthesis Assay (UDS) trials, the three (3) negative controls {the untreated cells control, F, and Dimethylsulfoxide (DMSO)} had negative values for Net Nuclear Gain (NNG) counts (<0). A positive control, 2-Aminofluorene (2-AF) was positive for induction of UDS; the mean NNG counts were 45.92 and 58.99 in the first and second assays, respectively, indicating assay validity. (i.e., hepatocytes were capable of metabolic activation and DNA repair). The positive control responses occurred at

toxic levels. UDS assay results for NNGs were in the range of -26 to -46, demonstrating a lack of UDS activity for the three (3) condensation products at concentrations greater than their solubilities in the test media. The results indicated that, under controlled laboratory conditions, the condensation products from the reaction of 1.4-Benzenediamine, N,N', mixed Ph and tolyl. derivs. with Dicyclopentadiene were negative for induction of UDS in rat hepatocytes at concentrations up to and greater than their solubilities. This assay demonstrated a lack of genetic activity in this mammalian DNA-repair test system.

Method: other: Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes on Test substance Condensation Products. The test substance, 1,4-Benzenediamine, N.N'-mixed Ph and tolyl. derivs., was reacted with Dicyclopentadiene in varying ratios, resulting in three condensation products. Each of these condensation products were subjected to independent in vitro unscheduled DNA synthesis (UDS) assays with hepatocytes from male Fischer 344 (F344/Crl) rats. All three (3) condensation products were tested at concentrations slightly above their limits of solubility in the tissue culture medium. Hepatocytes were exposed to test substances for 18-20 hours to allow bioactivation and DNA repair. The assay was based on the incorporation of 3H-thymidine into the hepatocyte's DNA during repair of DNA-damage. This incorporation was monitored by counting Net Nuclear Grains (NNG) formed on photographic emulsion placed on the cells adhering to glass slides. Criteria for a positive response included : (a) Significant increase in number of grains at two (2) levels of exposure above negative control levels, (b) A dose-responsiveness in grain counts up to toxic levels of exposure, and (c) At least one (1) value for NNG that is five (5) or above. A negative response is reported for NNG's that are <0, and an equivocal or inconclusive response are results that are 0<#<5.

Year: 1999 GLP: yes

Test substance: The test substance, 1,4-Benzenediamine, N.N'-mixed Ph and tolyl. Derivs. condensation products with Dicylopentadiene

Reliability: (1) valid without restriction
07-AUG-2000

(37)

5.6 Genetic Toxicity 'in Vivo'

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex:
Strain:
Route of admin.: Oral feed
Exposure period: 24 hours
Doses: 50 ug/ml and 10 ug/ml
Result: Negative. Negative under conditions of the assay
Method: other: Drosophila melanogaster (Fruit Fly) System
Year: 1979 GLP: no

Test substance: Nailax B (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000

(32)

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex:
Strain:
Route of admin.: Oral feed
Exposure period: 24 hours
Doses: 0.05 mg/ml and 0.63 mg/ml
Result: Negative. Negative under conditions of the assay
Method: other: Drosophilia SLRL Assay
Year: 1979 GLP: no

Test substance: Nailax (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000

(13)

Date: 22-Jan-2003
5. Toxicity ID: 68953-84-4

Type: Micronucleus assay
Species: Mouse Sex: male/female
Strain: CD-1
Route of admin.: i.p.
Exposure period: single dosing
Doses: 0, 250, 1250, 2500 mg/kg test chemical; 0.5 g/kg TEM (+ control)
Result: Negative. There were no statistically significant depressions in the PCE/NCE ratios in any groups of mice except for the 2500 mg/kg group at 48-hours sacrifice time ($p<0.01$) which was an indication that the test substance had reached the bone marrow and was toxic to erythrocytes.

Analysis of the micronucleus data for the groups treated with the test substance indicated that there were no statistically significant increases in the frequency of micronucleated PCEs. The test substance was judged negative (non-clastogenic) based on its inability to induce micronucleated PCEs.

Method: OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"

Nine (9) groups of mice (CD-1) were acclimated to laboratory conditions for 25-days prior to initiation of the study. The mice were randomized by body weight and assigned to groups using a computer-generated random number list.

Each group of mice was comprised of ten (10) animals(five (5) males/five (5) females). Each mouse received a single interperitoneal dose at 10 mL/kg of body weight. The test substance at dose levels of 250, 1250, and 2500 mg/kg was administered to three (3) groups of mice which were sacrificed at 24-, 48-, and 72-hours post dose. Concurrently, the negative control, Dimethylsulfoxide (DMSO)/corn oil, was administered, as dose volume of 10 mL/kg of body weight, to three (3) groups of mice. A group of these mice were included in each sampling time. The positive control, Triethylenemelamine at 0.5 mg/kg, was administered to one (1) group of mice and sacrificed at 24-hours post dose.

All mice were sacrificed and their femurs were removed. Their bone marrow was removed by flushing. Smears were made of the suspended cells.

One (1) thousand young erythrocytes were evaluated for a change of ratio of polychromatic erythrocytes (PCE) to normochromatic cells (NCE).

Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
04-AUG-2000

(18)

Type:	Other: 32P Postlabeling Assay for Detection of Adduct Formation in Rat DNA
Species:	rat Sex: male/female
Strain:	other: Fischer 344/N TacfBR
Route of admin.:	Gavage
Exposure period:	7 days
Doses:	0., 0.3, 1.0, and 3.0 g/kg/bw
Result:	Negative. Under conditions of the study, the test substance did not induce DNA-adducts in the liver and urinary bladder DNA of rats.
Method:	Other: 32P Post-Labeling Assay for DNA Adduct Formation
Year:	1995 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)
Remark:	The purpose of the study was to determine the potential of WINGSTAY 100 (mixed diaryl-p-phenylenediamines)to bind covalently to liver and urinary bladder DNA of male and female rats after in vivo administration of WINGSTAY 100.
Result:	Under conditions of the study, the test substance did not induce DNA-adducts in the liver and urinary bladder DNA of rats.
Reliability:	(1) valid without restriction
07-AUG-2000	

(4)

5.7 Carcinogenicity

Species:	rat	Sex: male
Strain:	Fischer 344	
Route of admin.:	oral feed	
Exposure period:	38 weeks	
Frequency of treatment:	Daily	
Post. obs. period:		
Doses:	1900 ppm	
Result:	Negative. The test substance exerted toxicity to the erythropoietic system, but there was an absence of tumor initiating or promoting activity.	

Control Group: yes, concurrent vehicle
Method: other: Accelerated bioassay (ABA)

The accelerated bioassay (ABA) was conducted on male F344 rats for 38 weeks. The target sites chosen for the ABA were liver and urinary bladder and the dose of the test substance was 1900 ppm as previously established to be a toxic dose. The liver tumor initiator was Diethylnitrosamine (DEN) and the urinary bladder initiator was N-Butyl N(4-hydroxybutyl)nitrosamine(BBN). The initiators, which included the test substance as a possible initiator, were administered during the first 14-weeks followed by the promoters. The promoters, Phenolbarbital (PB) for the liver and Nitrilotriacetate (NTA)for the urinary bladder and the test substance as a possible promoter, were administered during last 24-weeks after the test substance. The study had 11 test groups, including a negative control. The critical comparisons for initiation activity were conducted between Group Three (3) (PB) and Group Six (6) (Test substance + PB) for the liver and Group Eight(8) (NTA) and Eleven (11) (Test substance + NTA) for the urinary bladder. The critical comparisons for promoting activities were conducted between Group Two (2)(DEN) and Group Five (5) (DEN + Test substance) for the liver and Group Seven(7) (BBN) and Group Ten (10) (BBN + Test substance) for the urinary bladder. There were 26- and 38-week sacrifices.

Once daily, clinical observations were made and on scheduled body weighing days, a thorough palpation was performed on all animals. Body weights were recorded weekly from the first week of dosing until scheduled sacrifice at 26-weeks, and every 2-weeks thereafter.

At the two (2) scheduled sacrifices, all animals were subjected to a complete gross postmortem examination, The liver and kidneys were weighed. Liver, urinary bladder, kidneys and any grossly observed change or lesions were sampled, fixed, processed, cut and stained for microscopic examination. Tissue samples were taken from each of the three (3) liver lobes. NBF was used to inflate the urinary bladder at necropsy. All animals found dead or those killed in extremis were submitted to a complete gross postmortem examination. No organ weights were taken. The mean number of neoplasms per animal, the biggest diameter of carcinomas (in mm), the average diameter of carcinomas (in mm), and the degree of severity of carcinomas were recorded.

In order to assess proliferation, separate liver and urinary bladder sections were fixed in NBF, were cut and stained for PCNA. Subsequently, they all were aquatinted according to the method described above.

Statistical analyses were performed on weekly body weights, final body weights, absolute and relative liver and kidney weights, tumor incidence and PCNA data using methods described above.

Year: 1996 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines) The test substance was prepared in an olive oil suspension and mixed with rodent diet NIH-07 for dosing.

Reliability: (1) valid without restriction

(2)

Species: rat Sex: male/female
Strain: Fischer 344
Route of admin.: oral feed
Exposure period: 52 weeks
Frequency of treatment: Daily
Post. obs. period: 12 weeks
Doses: 53, 310, 1900 ppm
Result: Negative. No test substance related deaths occurred, although the high dose of 1900 ppm caused a decrease in body weight gain and food consumption in both genders. Red blood cell mean corpuscular volume was significantly increased at 38-weeks, accompanied by a significant decrease in mean corpuscular hemoglobin concentration.

At 52-weeks, the red blood cell count and hemoglobin values were also significantly decreased in high dose animals of both genders. Total bilirubin and cholesterol were increased in high dose animals at 38- and 52-week sacrifices. During the 3-month recovery, hematology parameters, bilirubin and cholesterol returned to control values. Total protein was reduced in high dose animals of both genders, throughout the entire exposure and recovery periods. The test substance also produced increases in relative liver, spleen, heart, and kidney weights in high dose animals. Both genders of all test substance groups exhibited significant increases in urothelial cell proliferation (measured by PCNA)and adaptive hyperplasia. No regenerative hyperplasia, prenoplasia, or neoplasia were present. There were microscopic evidence of extramedullary erythropoiesis in the spleen and liver of high dose animals in both genders; otherwise, no other pertinent microscopic findings were evident. The test substance exerted toxicity to the erythropoietic system, but displayed no carcinogenic activity.

Control Group: yes, concurrent vehicle

Method: other: One year study in male and female F 344 rats

The study used both genders of Fischer 344 (F344/N Tacf Br MPF) rats. There was a 38-week interim sacrifice in addition to 52-week, and 12-week post-exposure (recovery) sacrifice periods. The high dose in the study (1900 ppm) was the maximum tolerated dose identified in subchronic studies, in which there was no observable gender difference.

Once daily, cage side clinical observations were made, and on days scheduled for body weighing, a thorough body palpation was performed. Body weights were recorded one (1) week prior to initiation of exposure, weekly for weeks 1-13, and once every two (2) weeks thereafter. Food consumption was measured for weeks 1-13, and once every two (2) weeks thereafter. Indirect ophthalmoscopy was performed on all animals prior to exposure and during week-52.

During the three (3) sacrifices (at 38-, 52-, and 64-weeks), Five (5) rats/group/gender were used for hematology, clinical chemistry, and urinalysis. At scheduled sacrifices, all animals were subjected to a complete postmortem examination. Key organs were weighed and the tissues fixed in neutral buffered formalin (NBF), processed, cut, and stained with H&E. Tissue samples were taken from each of the three (3) liverlobes. NBF was used to inflate the urinary bladder at necropsy. All animals found dead and those killed in extremis were submitted to a complete gross postmortem examination. For these, no organ weights were taken, but all grossly observed changes and all key tissues were examined microscopically.

To assess cell proliferation, separate liver, urinary bladder and kidney sections were fixed in NBF, cut, and stained for proliferating cell nuclear antigen (PCNA). The quantitation of PCNA-positive nuclei in the immuno-stained sections of these tissues, was performed from 38-, 52-, and 62-week sacrifices. Next, the proliferation index (PI) for the liver, urinary bladder, and kidney for each animal was calculated, representing the percentage of PCNA-positive nuclei out of the total number of hepatocellular, urothelial, or tubular nuclei counted. The results were subjected to appropriate statistical analysis.

Statistical analysis was performed on weeking body weights, food consumption data, absolute and relative organ weights, hematology, clinical chemistry, urinalysis, and PCNA data.

Year: 1996 GLP: yes

Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines) The test substance was prepared in an olive oil suspension and mixed with rodent diet NIH-07 for dosing.

Reliability: (1) valid without restriction

(3)

5.8 Toxicity to Reproduction

Type: Two generation study
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure Period: F0 exposed during 10 weeks premating, 2 weeks of mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 males and females exposed for 10 weeks prior to mating.
Frequency of treatment: Daily
Premating Exposure Period:
male: 10 weeks
female: 10 weeks
Duration of test: 9 months
Doses: 0, 120, 400 or 1500 ppm.

Control Group: yes, concurrent no treatment

Method: OECD Guide-line 416 "Two-generation Reproduction Toxicity Study"

This study was designed in compliance with EPA GLP and USEPA FIFRA guidelines. Dose levels were established from a Range finding study at Research Triangle Institute which employed dietary levels of 120, 1900, and 5700 ppm of WINGSTAY 100 (mixed diaryl-p-phenylenediamines). The top level was lethal to dams and offspring, 1900 ppm induced one nonviable litter in 9 total, and thus, the top dose for the definitive study was decreased by 20% to assure high viability in test group. No effects were seen at 120 ppm.

This study used 30 Sprague Dawley rats/sex/dose (F0) exposed to diets containing 0, 120, 400 or 1500 ppm WINGSTAY 100 during 10 weeks premating, 2 weeks mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 litters were culled to 10 each at 4 days postnatal (PND) 30 other F1 males and females/group chosen for pairing, and fed WINGSTAY 100 as above for 10 weeks prior to mating. After mating/gestation of F1, the resulting F2 rats were delivered, and maintained through weaning period (to PND 21). Weekly body weights (BWs) and food consumption (FC), and daily clinical observations were recorded. Necropsies and histopathology (primary kidneys) were performed on selected rats from each sex/group/generation (all F0 and F1 dams at PND21, three F1 and F2 pups/test group at PND21). Remaining F1 and F2 rats were euthanized without examination. Data were collected on vaginal cytology, mating, pregnancy, litter, and pup parameters.

Remark: WINGSTAY 100 induced dystocia (difficult deliveries) in pregnant rats which may have led to prolonged gestation and increased perinatal deaths, decreased live births, and increased pup weights. In addition, polycystic lesions were observed at all dose levels. Prolonged gestation has previously been associated with the WINGSTAY component DPPD, and polycystic kidneys were observed in DPamine-treated rats. Based upon adult toxicities, reproductive and offspring endpoints, there was no NOEL for WINGSTAY 100 in this study.

Result: High dose females had decreased Body Weights (BWs) relative to other test groups throughout majority of study period. Mortality during gestation/lacation were: F0 dams- 0 in 24 pregnancies, 0/27, 3/24, 4/25; F1- 0/22, 0/23, 1/22, 1/24. Numbers of pregnancies with no live births: F0- 0, 1, 1, 10; F1- 0, 1, 1, 2. Gestational length: F0- 22.2 days, 22.4 days, 22.8*, 23.5*; F1- 22.2, 22.8*, 23.1*, 23.2* (* = statistically significant). The number of live pups/litter: F0-15.6, 14.1, 11.9, 7.6*; F1- 15.6, 13.7, 13.3, 10.8*. Pups weights (g) on PND 0: F0- 6.38, 6.79*, 6.93*, 6.63*; F1- 6.32, 6.89*, 6.99*, 6.63*.

WINGSTAY 100-related kidney lesions were observed grossly (as white or clear cysts) and microscopically (polycyctic findings with variable severity): F0 adults-males 0/0, 0/0, 0/0, 0/1 and females 0/0, 0/0, 0/2, 3/9; F1 weanlings-males 0/23, 1/25, 8/20, 10/11 and females 0/22, 5/26, 7/18, 11/11; F1 adults-males 0/30, 5/30, 10/30, 21/30 and females 0/30, 2/30, 1/30, 18/30; F2 weanlings-males 0/60, 3/64, 6/19, 15/16 and females 0/60, 5/64, 8/19, 15/15. The severity of kidney lesions were also dose related.

Year: 2000 GLP: yes

Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines) The test substance was prepared in an olive oil suspension and mixed with rodent diet NIH-07 for dosing.

Reliability: (1) valid without restriction
11-FEB-2001

(36)

Type: Two generation study
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure Period: F0 exposed during 10 weeks premating, 2 weeks of mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 males and females exposed for 10 weeks prior to mating.
Frequency of treatment: Daily
Premating Exposure Period:
male: 10 weeks
female: 10 weeks
Duration of test: 9 months
Doses: 0, 120, 400 or 1500 ppm.

Control Group: yes, concurrent no treatment

Method: Other: Derivation of Benchmark Dose from 2-Generation Rat Study
Test Substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Bench Mark Responses (BMR) are estimations of doses inducing a discrete toxic response in a test population at an incidence within the range of 1-10%. The Bench Mark Dose (BMD) is represented as the 95% lower confidence limit (LCL) for a BMR, or as a Most Likely Estimate (MLE). In this project, data from the 2-generation reproduction study in rats on Wingstay 100 (RTI #65C-6429-400)(36) chosen for analyses were the (1) polycystic kidney lesions in F1 male adults and F1 female weanlings, and (2) gestational lengths (days) for F1 pregnant females.

Data for these endpoints at the 3 dose levels employed in the study were subjected to various analyses including Gamma, Multistage, Quantal Linear, Weibull, Probit, Logistic, and Quantal Quadratic (for quantal data – polycystic kidneys), and Power, Linear, and Polynomial models (continuous data – gestational lengths). Estimations were also made to derive "best fit" information for each model run. The methodology employed was according to the "Benchmark Dose Technical Guidance Document" (1996), EPA/600/P-96/002A.

Results: Most Likely Estimate (MLE) and 95% Lowest Confidence Limit (LCL) values were derived for the most sensitive toxic endpoints (observed graphically). The models that "best fit" polycystic data for F1 male adults and F1 female weanlings were the quantal linear and multistage procedures. The BMD 10% values (EPA default for quantal data) derived for F1 male adults are 7 mg/kg-day (LCL) and 9.3 (MLE), and for F1 female weanlings, the values are 3.7 and 6.0 mg/kg-day, respectively. The prolongation of parturition analysis for F1 females indicated that none of the models produced a good fit although there was good agreement amongst the 3 models tested, giving BMD 5% estimations of 160 (LEL) and 226 (MLE) mg/kg-day for this endpoint.

The Bench Mark Dose (10% incidence) developed for the the most sensitive endpoint (polycystic kidneys in F1 female weanling rats) in the 2-generation rat dietary study was 3.7 (95% Lower Confidence Limit) and 6.0 (Most Likely Estimate) mg/kg-day. These numbers are below the lowest exposure levels (and LOEL) found in the 2-generation study, and thus pose plausible estimates of a 10% incidence rate for this endpoint. These calculations provide a credible low dose benchmark that can be used as a basis for safety assessments in exposed populations.

(40)

5.9 Developmental Toxicity/Teratogenicity

Species:	rat	Sex: female
Strain:	Sprague-Dawley	
Route of admin.:	gavage	
Exposure period:	10 days	
Frequency of treatment:	Dosed on days 6-15 gestation	
Duration of test:		
Doses:	0, 20, 70, 200 mg test material in 5 ml corn oil/kg	
Control Group:	yes, concurrent vehicle	
NOAEL Maternalt.:	70 mg/kg bw	
NOAEL Teratogen.:	<= 200 mg/kg bw	
NOAEL Fetal:	70 mg/kg bw	
Method:	OECD Guide-line 414 "Teratogenicity"	

Preliminary trials in 8 rats/group indicated that 600 mg/kg was lethal to 50% of maternal rats while 200 mg.kg caused decreased body weights in maternal and fetal animals. There were no effects at 20 or 70 mg/kg. Consequently, 200 mg/kg was selected as the top (high) dose in the definitive study, Confirmation of the test dose solutions were confirmed analytically.

The definitive study used 25 inseminated female rats per test group (0, 20, 70, and 200 mg of test substance/kg doses in five (5) mL corn oil/kg). The animals were dosed on Days 6-15 gestation. Body weights, food consumption, liver weights, clinical changes, pregnancy rates, and corpora lutea counts were followed along with numerous fetal parameters. All fetuses were weighed, sexed, and assessed for external and visceral abnormalities. One (1) half of the fetuses were examined for skeletal abnormalities while the second half were subjected to cranial bone assessments.

Remark: Administered in 5 ml corn oil/kg by gavage

Result: The test substance induced no lethality. Deficits were seen in maternal body weights (Day-12 and body weight change from Day-6 to Day-15) and food consumption (during treatment period) at the highest dose only (200 mg/kg). Pregnancy rates, litter sizes, number of live fetuses, uterine implantation, and all gestational parameters were unaffected by chemical treatment. There was a linear trend towards lower body weights in fetuses with increasing doses (approximately 5% decrease in 200 mg/kg group). Assessment of cranial, skeletal, visceral, and external appearance discerned no compound-related abnormalities (malformatiuons or variations) according to established criteria. The test material produced minimal effects (body weight) to maternal rats from oral dosing of 200 mg/kg during pregnancy. There was no induction by the test chemical of birth defects (major or minor) in fetal animals.

22-Jan-2003
5. Toxicity ID: 68953-84-4

Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (1) valid without restriction
08-AUG-2000

(22)

Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure period: Varied, see method
Frequency of treatment: Varied, see method
Duration of test:
Doses: 2500 ppm
Control Group: yes, concurrent vehicle
Method: other: Mechanistic Study

The toxicity of the test substance to maternal and 1st generation offspring was evaluated by exposing CD (Sprague-Dawley) rats to fixed dietary concentrations of 2500 ppm during different time periods (i.e. exposures during prebreed, mating, gestation, and/or lactation). Five (5) Groups (20/sex/Group) were studied including: Group one (1)- Negative control; Group two (2)- Dietary test substance during prebreed and mating, exposures ended on gestation day (gd)-0; Group three (3)- Dietary test substance during gestation and lactation, exposures began on gd-0; Group four (4)- Dietary test substance during prebreed, mating, gestation, and lactation, the Positive control and; Group five (5)- Dietary test substance during prebreed, mating, gestation, and lactation, plus 600 ppm of iron gluconate in the drinking water for prebreed through lactation.

Males and females were paired within Groups (1:1) for the two-week mating period. Once a given female was found to be sperm positive {date designated as gestation day (gd)-0}, "her" male was euthanized and discarded. On the day of delivery (pnd-0), pups were counted, sexed, and weighed. On pnd-4, litters were culled to ten, counted, sexed, and weighed. On pnd-7, -14, and -21, pups were counted, sexed, and weighed. All pups were euthanized and one (1)/sex/litter necropsied on pnd-21. Dead pups on pnd-0 and -1 were examined macroscopically (necropsied) for polycystic kidneys. Female body weights and feed consumption were recorded weekly during prebreed, gestation, and postnatally. At necropsy on pnd-21, the maternal spleen, liver, and kidneys were weighed and retained in a fixative. Kidneys form Groups one (1) and five (5) were examined histopathologically.

22-Jan-2003
5. Toxicity ID: 68953-84-4

Blood sampling was performed ongestation day-21 and pnd-21 from all females (pregnant) by tail vein withdrawal. Blood sampling was performed on pnd-21 on the F1 offspring by withdrawal from the abdominal vena cava at sacrifice. The blood parameters assessed were: WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, Platelets, WBC Differential (to correct the RBC and WBC counts for Nucleated Red Blood Cells) and Methemoglobin. On gd-21, a second sample of blood was taken via tail vein from all pregnant females in all Groups, with plasma frozen for possible subsequent analysis for specific hormones. For Group three(3), any female who had not yet delivered by gestation day-23 had blood taken from the tail vein and plasma frozen. On pnd-21, the spleen, liver, kidneys, and heart from one(1) pup/sex/litter were weighed and retained in a fixative.The kidneys from all offspring were examined histologically. Statistical analysis included both parametric and nonparametric tests for continuous and discrete data.

Remark: The objectives of this study were to confirm and further characterize previously-observed effects following the test substance administration to pregnant rats. This study was designed (1) to determine the necessary and sufficient timing of exposure to maternal females at a fixed dietary concentration of the test substance to produce dystocia, prolonged gestation, and polycystic kidneys in offspring, (2) to determine whether the test substance results in demonstratable macrocytic anemia in maternal animals, (3) to determine if there is treatment-induced anemia and whether iron supplementation ameliorates or prevents the anemia, dystocia, and/or polycystic kidneys, and (4) to determine if F0 parental females exhibit polycystic kidneys due to dietary exposure to the test substance.

Result: F0 Males: The test substance intake over the prebreed period (Study Days 0-28) averaged 180 mg/kg/day for all three (3) exposed Groups {two (2), four (4), and five (5)}. Iron gluconate intake in Group five (5) averaged 56 mg/kg/day (Study Days-0 to 28). Clinical observations were found to be unrelated to compound administration.

F0 Females: The test substance intake averaged 187-192 mg/kg/day for Groups two (2), four (4) and five (5) during gestation days (gd)-0 to 28. Iron gluconate intake during gestational days-0 to 28 in Group five (5) averaged 53 mg/kg/day. Clinical observations during gestation included one (1) female found dead in Groups three (3) and four (4), alopecia predominantly in Groups four (4) and five (5), pale eyes and tail, pale (not otherwise specified) almost exclusively in Groups three(3), four (4) and five (5) (all exposed), pilorection in Groups three (3), four (4) and five (5), and delayed parturition in Groups three (3), four (4), and five (5).

The hematological profile of maternal rats on gestation day-21 found no evidence on macrocytic anemia in any groups.

REPRODUCTIVE/DEVELOPMENTAL: Gestational index (a measure of live litters relative to pregnant females) was significantly increased in Groups three (3) and four (4) but not in Group five (5). Male mating, fertility, and pregnancy indices were equivalent across all groups. Gestational length in days was significantly prolonged in Group three (3) (23.6+/-0.2), Group four (4) (23.8+/-0.2), and Group five (5) (23.5+/-0.2) relative to Control Group value (22.2+/-0.1) and the value in Group two (2) (22.3+/-0.1). Number of implantation sites per litter was significantly reduced in Group five (5). Percent of post implantation loss was significantly increased in Groups three (3) and four (4). Pups per litter were significantly reduced in Groups three (3), four (4) and five (5), and number of dead pups per litter were significantly increased in Groups three (3) and four (4). Weanling gross and microscopic findings were limited to hydronephrosis in Groups one (1) and two (2), gas in intestines in Group two (2), and gross evidence of polycystic kidneys in Groups three (3), four (4), and five (5). Maternal hematologic profiles at sacrifice (21 days after delivery) indicated statistically significant changes in most erythrocyte parameters. The white blood cell differential counts indicated changes (as percent of cells examined) as follows: increase in segmented neutrophils and decrease in lymphocytes only in Group four (4), with no treatment-related changes in the percentages of monocytes or eosinophils. Histopathologic assessment was performed on kidneys of all maternal rats in Groups one (1) and five (5). Polycystic kidneys were observed microscopically (but not macroscopically) in three (3) of 20 animals in Group five (5), with no polycystic kidneys observed in Group one (1).

The timing of exposure to the test substance with respect to pregnancy is an important determinant of toxicity. Exposure of F0 females to 2500 ppm of the test material during gestation is necessary and sufficient to produce dystocia (prolonged gestation).

It is necessary and sufficient to expose F0 dams during gestation and/or lactation to produce polycystic kidneys in the F1 offspring. Since no Groups were exposed only during gestation or only during lactation, it is not possible to further define how exposure timing affects this endpoint. There was no demonstrable macrocytic anemia in gestation day-21 (gd-21) F0 dams in any treatment Group, but at post delivery day-21 (pnd-21), F0 mothers exposed prior to and during mating, gestation, and lactation were anemic. The F1 offspring at pnd-21 did not consistently display evidence of macrocytic anemia. Iron supplementation did not affect pnd-21 maternal anemia, dystocia, or incidence/severity of polycystic kidneys in the F1 offspring. However, perinatal survival of the offspring was affected. Microscopic, but not macroscopic evidence of polycystic kidneys was found in 15 percent of dams treated prior to and during mating, gestation, and lactation (with iron supplementation). Controls had neither macroscopic nor microscopic indications of polycystic kidneys. Exposure of animals to the test substance prior to and during mating {Group two (2)} did not appear to result in adverse affects to offspring. Furthermore, exposure during the prebreed/mating periods did not increase the affects produced from gestation/lactation exposures only.

Year: 2000 GLP: no
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although this study was not conducted to GLP, the test parameters used were based on a sound scientific design.
09-AUG-2000

(15)

5.10 Other Relevant Information

Type: other: A Photoirritation Study in Rabbits
Method: US FDA test guidelines and GLPs.
Result: UV light did not enhance the skin irritation response of the test substance in rabbits, and therefore is not considered to be a photo-irritant.

Test condition: Albino rabbits (4 females,4 males) were shaved in the dorsal portion of the animals trunk. One day later, 0.5 g of test material was placed onto 2 skin site of 3 male and 3 female rabbits. 0.5 ml of Oxsoralen lotion was similarly applied to 1 male and 1 female rabbit. After 2-hour skin contact exposure period, the gauze patches were removed from the animals' right sides and the left side sites were covered with aluminum foil to prevent light exposure. All animals were exposed to UVA light for 40 minutes. Following light exposures, the gauze patches were reattached for additional 21 hours.

Skin sites were scored according to Draize procedures at 25, 48 and 72 hours plus 7 days following cessation of chemical exposure.

Reliability: (1) valid without restriction

(1)

other: Mechanistic

Method: Dietary WINGSTAY 100 (mixed diaryl-p-phenylenediamines) induced dystocia and delayed parturition with associated maternal deaths in pregnant rats in a 2-generation reproduction study. This mechanistic study was designed to assess exposure conditions necessary to induce these findings, and the role of possible iron deficiency. Female rats were exposed to 2500 ppm of WINGSTAY 100 in the diet as follows:

Group 1- 0 ppm for 12 week study (negative control)
Group 2- Exposed 4 weeks prebreed plus 2 weeks mating
Group 3- Exposed 3 weeks gestation plus 3 weeks lactation
Group 4- Exposed 4 weeks prebreed, 2 weeks mating, 3 weeks gestation, 3 weeks lactation (positive control)
Group 5- Positive control plus iron supplementation (600 ppm iron gluconate in drinking water)

Females (20/group) were mated with males with comparable dietary exposures. Following confirmed mating, males were sacrificed without further assessment. Rats were subjected to daily observations, weekly Body Weights (BWs), and feed and water consumptions. Maternal F0 rats were bled on gestational day 21 prior to delivery and post delivery day 21. A sample of plasma was frozen from the gestation day 21 bleeding for possible future endrocrine assessments. F1 rats were bled on day 21 post natal. Samples were subjected to standard hematology and metHgb assays. Major organ weights were determined. Observations were made during reproductive, gestational, and postnatal periods of the study. Necropsies with organ weights determinations were performed on all surviving F0 and F1 rats 21 days post delivery. Microscopic exams were performed on gross lesions in F0 rats, and on kidneys of F0 and F1 animals.

Remark: The study confirmed results in a 2-generation reproduction rat study that demonstrated dietary WINGSTAY 100 induces dystocia, delayed parturitition, and an associated decrease in pup survival at birth.

These findings have earlier been associated with DPPD and DPA according to available literature. The effects in Group 3, but not Group 2 indicate that chemical exposure during gestational period is essential
for the dystocia and delayed parturition observed. Since Group 3 included exposure during lactation, it is uncertain whether gestational exposure alone would induce the polycyctic kidneys in offspring. Pre-gestational exposure did not enhance the effects attributed to gestational WINGSTAY 100 ingestion. Finally, although iron supplementation had no apparent impact on blood parameters, it did decrease the number of stillbirths without impacting other reproductive or litter endpoints.

Result: Body weights and feed consumption for F0 rats were reduced relative to negative controls, possibly as a result of decreased palatability of the WINGSTAY 100-containing diet. One (1) Group 3 female died on gestation day 19, and one (1) Group 4 rat on gestation day 24. Due to dead litters, additional Groups 3 and 4 dams were euthanized. Other clinical observations included alopecia and pale appearance (eyes, tails and ears) in Groups 2-5 throughout study. There were no indications of RBC, WBC, or Hgb changes ascribed to WINGSTAY 100 exposure. RBC size distribution width was decreased, demonstrating lack of macrocytic changes. The fertility indices (number of pregnancies/number of matings) were 79, 74, 90, 79, and 71%. Gestational indices (number of females with live litters/number of pregnancies) were 100, 93, 65, 71, and 100%, and the gestational lengths were 22.2, 22.3, 23.6, 23.8, and 23.5 days (Groups 3-5 were significantly delayed). Litter effects included stillbirths (3, 1, 45, 46, and 10% of total pups delivered), decreased pup survival (13, 13, 6, 7, and 8 live pups/litter) on post natal day 0 and 10, 10, 6, 8, and 7 on day 21. Relative liver and heart weights were increased for Groups 3-5 F1 pups. Gross observations included polycyctic kidneys in male and female F1 Groups 3-5 pups, confirmed microscopically in part as dilatation in the papillary region. Rates of these renal lesions were in excess of 80% in both male and female rats. Microscopic results for the F0 females included a 15% incidence of polycyctic kidneys in Group 5 and
none in Group 1. The other groups were not examined microscopically.

Date: 2/7/00
Test Substance: Wingstay 100 (mixed diaryl-p-phenylenediamines

Reliability: (1) valid without restriction

(14)

(1) A Photoirritation Study in Rabbits with WINGSTAY 100, Report # 3097.28 to The Goodyear Tire & Rubber Company, August 24, 1995

(2) 38-Week Accelerated Bioassay (ABA) of WINGSTAY 100 in Rats, American Health Foundation, 1996

(3) 52-Week Chronic Feeding Study of WINGSTAY 100 in Rats with 12-Week Recovery. Report # AHF R1695, American Health Foundation, 12/16/1996

(4) American Health Foundation, 32P Postlabeling Assay for Detection of Adduct Formation by WINGSTAY 100 and R-59 in Rat Liver and Urinary Bladder DNA, AHF Study No. 93-63 to The Goodyear Tire & Rubber Company, 1995

(5) American Health Foundation, Preliminary Oral 3-Week Range-Finding Study for Administration of WINGSTAY 100 and R-59 in Male and Female Fischer 344 Rats, AHF Study R-1626 to The Goodyear Tire & Rubber Company, 1994.

(6) Bayer AG Data

(7) Bayer AG, Report No. 19778, December 10, 1990.

(8) Bayer AG, Report Number 19940, February 4, 1991.

(9) Bayer AG, Unpublished Data, July 2, 1992

(10) Bioaccumulation Test of Wingstay 100 in Carp, Report Study Number 43172, Kurume Research Laboratories/CITI, 12/18/1998

(11) Four-Week Dietary Study of WINGSTAY 100 in Fischer 344 Rats, Report # AHF R1664, American Health Foundation, 1/31/96

(12) Litton Bionetics, Inc., Balb/3T3 In Vitro Transformation Assay of NAILAX, Genetics Assay No.5419 to The Goodyear Tire & Rubber Company, 1981.

(13) Litton Bionetics, Inc., Drosophilia SLRL Assay with Nailax to The Goodyear Tire & Rubber Company, 1979.

(14) Mechanistic Study of WINGSTAY 100, Report #: 65C-6429-500, Research Triangle Institute, 2/7/00

(15) Mechanistic Study of Wingstay 100, Report Study # RTI 65C-6429-500, Research Triangle Park, February 11, 2000

(16) Pharmakon USA, Report # Ph301-GY-001-93 to The Goodyear Tire & Rubber Company, 1993

(17) Pharmakon USA, Report # Ph301-GY-004-93 to The Goodyear Tire & Rubber Company, 1994

(18) Pharmakon USA, Report # Ph309-GY-001-93 to The Goodyear Tire & Rubber Company, 1993.

(19) Pharmakon USA, Report # Ph320-GY-001-93 to The Goodyear Tire & Rubber Company, 1993.

(20) Pharmakon USA, Report # Ph402-GY-001-93 to The Goodyear Tire & Rubber Company, 1993.

(22) Reseach Triangle Research, Developmental Toxicity Evaluation of WINGSTAY 100 Administered by Gavage to CD (Sprague-Dawley) Rats, Report # 65C-5962-100/200 to The Goodyear Tire & Rubber Company, July 11, 1995.

(23) Ricerca, Inc., Biodegradation Study of a Rubber Antioxidant, Document Number 6011-94-0037-BC-001 to The Goodyear Tire & Rubber Company, 1994

(24) Springborn Laboratories, A Dermal Sensitization Study in Guinea Pigs with WINGSTAY 100-Maximization Design, Report # 3097.30 to The Goodyear Tire & Rubbers Company, August 24, 1995.

(25) Springborn Laboratories, A Primary Eye Irritation Study in Rabbits with WINGSTAY 100, Report # 3097.27 to The Goodyear Tire & Rubber Company, August 24, 1995.

(26) Springborn Laboratories, A Primary Skin Irritation Study in Rabbits with WINGSTAY 100, Report # 3097.26 to The Goodyear Tire & Rubber Company, August 24, 1995.

(27) Springborn Laboratories, An Acute Toxicity Study in Rabbits with WINGSTAY 100 (Limit Test), Report # S94-001-3097.29 to The Goodyear Tire & Rubber Company, August 24, 1995.

(28) Springborn Laboratories, WINGSTAY 100-Acute Toxicity to Daphnids Under Flow-Through Conditions, Report # 96-1-6328 to The Goodyear Tire & Rubber Company, June 26, 1996.

(29) Springborn Laboratories, WINGSTAY 100-Determination of n-Octanol/Water Partition Coefficient, Report # 95-9-6103 to The Goodyear Tire & Rubber Company, December 12, 1995

(30) Springborn Laboratories, WINGSTAY 100-Prolonged (14-day) Acute Toxicity to Common Carp Under Flow-Through Conditions, Report # 96-2-6362 to The Goodyear Tire & Rubber Company, June 28, 1996

(31) Springborn Laboratories, WINGSTAY 100-Toxicity to the Freshwater Green Alga, Report # 96-4-6454 to The Goodyear Tire & Rubber Company, July 2, 1996.

(32) The Goodyear Tire & Rubber Company, Biological Effects of Nailax B in a Drosophilia melanogaster (Fruit Fly) Test System, 1979.

(33) The Goodyear Tire & Rubber Company, DNA Damage by WINGSTAY 100 Lot 48-3012 in the E. coli Pol A1- Assay, 1980.

(34) The Goodyear Tire & Rubber Company, WIGSTAY 100, Material Safety Data Sheet, 2001.

(35) The Goodyear Tire & Rubber Company, WINGSTAY 100, Material Safety Data Sheet, 2001.

(36) Two-Generation Reproductive Toxicity Evaluation of WINGSTAY 100 Administered in the Feed to CD (Sprague-Dawley) Rats, Report #: 65C-6429-400/200, Research Triangle Institute, 12/8/00.

(37) Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes on Wingstay 100 Condensation Products RWC-7703, RWX-7704, and RWC-7706, American Health Foundation, December 20, 1999

(38) WINGSTAY 100-Prolonged (14-Day) Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) Under Flow-through Conditions, Report # 96-11-6700, Springborn Laboratories, 2/21/97.

(39) Wisconsin Alumni Research Foundation, Acute Toxicity of WINGSTAY 100 to The Goodyear Tire & Rubber Company, 1959.

(40) The Sapphire Group, Derivation of Benchmark Dose from 2-Generation Rat Study,2001.

201-14611

NCIC HPV
Sent by: Mary-Beth Weaver

07/21/2003 10:13 AM

To: NCIC HPV, moran.matthew@epa.gov
cc:
cc:
Subject: rapa: revised HPV submission for the Substituted p-Phenylenediamines category

Anne_LeHuray@americanchemistry.com on 07/18/2003 03:12:10 PM

To: Oppt.ncic@epamail.epa.gov, Rtk Chem/DC/USEPA/US@EPA
cc: Leslie Scott/DC/USEPA/US@EPA, Jim_Keith@americanchemistry.com

Subject: rapa: revised HPV submission for the Substituted p-Phenylenediamines category

Please find attached a ZIP file containing a cover letter and the revised Category Justification and Testing Rationale and the revised robust summaries for each of the five sponsored chemicals and the two supporting chemicals in the Substituted p-Phenylenediamines category. These documents are revisions of documents submitted to EPA on December 18, 2001. In preparing the revised Category Justification and Testing Rationale and the revised robust summaries, comments received from EPA (dated November 5, 2002) and from Environmental Defense (dated May 15, 2002) have been considered.

Hard copies of this submission have been sent to the Merrifield post office address.

(See attached file: substituted_p_phenylenediamines_071703.zip)

Please let me know if there are any difficulties with this transmission.

Anne P. LeHuray, Ph.D.
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substituted_p_phenylenediamines_071703.zip

201-14611

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council

July 17, 2003

Via US Mail and e-mail

Marianne Lamont Horinko
U.S. Environmental Protection Agency (EPA)
P.O. Box 1473
Merrifield, VA 22116

Re: Rubber and Plastic Additives (RAPA) Panel
HPV Chemical Challenge Program Submission
Substituted p-Phenylenediamines (PPD) Category
Revised Category Justification and Testing Rationale

Dear Ms. Horinko:

The RAPA Panel of the American Chemistry Council is pleased to submit the revised test plan and robust summaries for the Substituted p-Phenylediamines category, which includes five of the 37 chemicals RAPA is voluntarily sponsoring in the Program. The RAPA Panel includes the following member companies: Alco Chemicals; Bayer Polymers LLC.; Ciba Specialty Chemicals Corporation; Crompton Corporation; Eliokem, Inc.; Flexsys America L.P.; The Goodyear Tire & Rubber Company; The Lubrizol Corporation; Noveon, Inc.; and, R.T. Vanderbilt Company, Inc.

In this submission, please find the revised *Category Justification and Testing Rationale* for the category *Substituted p-Phenylenediamines*. Five chemicals in the category are sponsored in the Program, as listed in the following table:

RAPA Panel Substituted p-Phenylenediamines Category Chemicals Sponsored in the US HPV Chemical Challenge Program	
CAS Number	**Compound Name**
101-96-2	p-Phenylenediamine, N,N-di-sec-butyl
3081-14-9	p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl)
68953-84-4	1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives
3081-01-4	p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl
15233-47-3	p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl,

Data for two additional chemicals in the category, listed in the table below, are used to support the conclusions reached for the category.

RAPA Panel Substituted p-Phenylenediamines Category Additional Chemicals in the Category	
CAS Number	**Compound Name**
101-72-4	p-Phenylenediamine, N-Isopropyl-N'-phenyl-,
793-24-8	p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl

In addition to the revised *Category Justification and Testing Rationale*, please also find attached revised robust summaries contained in IUCLID-formatted documents for each of the five sponsored chemicals and the two supporting chemicals in the category. These documents are revisions of documents submitted to EPA on December 18, 2001. In preparing the revised *Category Justification and Testing Rationale* and the revised robust summaries, comments received from EPA (dated November 5, 2002) and from Environmental Defense (dated May 15, 2002) have been considered.

This submission is also being sent electronically to the following e-mail addresses:

Oppt.ncic@epa.gov
Chem.rtk@epa.gov

If you require additional information, please contact the RAPA Panel's technical contact, Dr. Anne P. LeHuray at (703) 741-5630 or *anne_lehuray@americanchemistry.com*.

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

Attachments

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Substituted p-Phenylenediamines
Category Justification and Testing Rationale (Revised)

CAS Nos. 101-96-2, 3081-14-9, 3081-01-4, 15233-47-3, and 68953-84-4
(+ SIDS Chemicals 101-72-4 and 793-24-8 for data purposes)
Rubber and Plastic Additives Panel of
The American Chemistry Council
July 2003

List of Member Companies in the Rubber and Plastic Additives Panel

The Rubber and Plastic Additives Panel of the American Chemistry Council include the following member companies: Alco Chemicals; Bayer Polymers LLC.; Ciba Specialty Chemicals Corporation; Crompton Corporation; Eliokem, Inc.; Flexsys America L.P.; The Goodyear Tire & Rubber Company; The Lubrizol Corporation; Noveon, Inc.; and, R.T. Vanderbilt Company, Inc.

Executive Summary

The American Chemistry Council's Rubber and Plastic Additives Panel (RAPA), and its member companies, hereby submit the revised *Category Justification and Testing Rationale* for the Substituted p-Phenylenediamines category of chemicals under the Environmental Protection Agency's High Production Volume (HPV) Challenge Program. This document and the accompanying revised robust summaries are revisions of documents submitted by the RAPA Panel in support of the category on December 18, 2001, and reflect consideration of comments received from EPA (dated November 5, 2002) and from Environmental Defense (dated May 15, 2002).

As discussed in the report that follows, Substituted p-Phenylenediamines (PPD), which are used as antidegradants in rubber, fuel additives, or in monomer distillation, are defined as phenylenediamines with various substitutions. These uses require stability at high temperatures, low biodegradation and very low water solubility and low vapor pressure. In consideration of animal welfare concerns to minimize the use of animals in the testing of chemicals, the Panel has conducted an extensive literature search for available data, published and unpublished. It has also performed an analysis of the adequacy of the existing data. Further, it developed a scientifically supportable category of related chemicals and used structure-activity relationship information to address certain data requirements. Existing data for members of this category indicate that they are of moderate to high toxicity in the aquatic environment, and of low concern for mammalian toxicity. No testing is proposed for the chemicals that constitute the Substituted p-Phenylenediamines category for the purposes of the HPV Program.

Substituted p-Phenylenediamines category

Relying on several factors specified in EPA's guidance document on "Development of Chemical Categories in the HPV Challenge Program," in which use of chemical categories is encouraged, the following closely related chemicals constitute a chemical category:

Substituted p-Phenylenediamines

Alkylated Phenylenediamines

p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)

4-Aminodiphenylamine Derivatives

p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3)

The goal of developing a chemical category is to use interpolation and/or extrapolation to assess chemicals rather than conducting additional unnecessary testing with specific consideration of animal welfare concerns to minimize the use of animals in the testing of chemicals.

Structural Similarity. A key factor supporting the classification of these chemicals as a category is their structural similarity (see Figure 1). All materials in this category are phenylenediamines with various substituent groups that are always in the *para* position of the aromatic ring. The substituent groups may be all alkyl, all aryl, or mixed alkyl/aryl.

Similarity of Physicochemical Properties. The similarity of the physicochemical properties of these materials parallels their structural similarity. All are highly-colored (dark brown, purple, reddish or black) solids or semi-viscous liquids intended for use as antidegradants in dark-colored or black finished rubber articles or functional fluids. The use of these materials requires that they be stable under high temperatures. Their low volatility is due to their low vapor pressure, semi-viscous or solid form. The existing information for these materials indicates that they have very low water solubility and high flash points.

Fate and Transport Characteristics. Members of this category have been tested and shown not to be readily biodegradable via CO_2 evolution, but they are susceptible to both hydrolysis and photodegradation. Additional data collection efforts are not proposed. These materials have been shown not to partition to water or air if released into the environment due to their low water solubility and low vapor pressure; as a result additional computer-modeled environmental partitioning data is not necessary for the members of this category for the purposes of the HPV Program.

Toxicological Similarity. Review of existing published and unpublished test data for Substituted p-Phenylenediamines shows the aquatic and mammalian toxicity among the materials within this category are similar.

Aquatic Toxicology. Data on acute fish toxicity, acute invertebrate toxicity, and algae toxicity were reviewed. The Substituted p-Phenylenediamines, in general, are very toxic to aquatic organisms. Additional testing is not proposed for these materials for the purposes of the HPV Program.

Mammalian Toxicology - Acute. Data on acute mammalian toxicity were reviewed, and the findings indicate a low concern for acute toxicity for all materials. Data are available for most members of the category indicating that the category has been well tested for acute mammalian effects. Therefore, no additional acute mammalian toxicity testing is proposed for the purposes of the HPV Program.

Mammalian Toxicology - Mutagenicity. Data from bacterial reverse mutation assays, *in vitro* and *in vivo* chromosome aberration studies, as well as additional supporting *in vitro* and *in vivo* genetic toxicity studies were reviewed, and the findings indicate a low concern for mutagenicity. Data are available for several members of the category or close structural analogs, and these data can be bridged to the other members of the category. Therefore, the category has been adequately tested for mutagenicity to meet the requirements of the HPV Program; therefore, no additional mutagenicity testing is proposed.

Mammalian Toxicology – Repeated Dose Toxicity. Data from repeated-dose toxicity studies were reviewed and sufficient data are available to satisfy the repeated dose toxicity requirements of this category through bridging to members without test data, such that additional testing is not proposed for these materials for the purposes of the HPV Program.

Mammalian Toxicology - Reproductive and Developmental Toxicity. There are several adequate reproductive/developmental studies for members of the Substituted p-Phenylene diamines category. Again, existing study data and results can be bridged to other category members, such that additional testing is not proposed for the purposes of the HPV Program.

Conclusion. Based upon data reviewed for the HPV program, the physicochemical and toxicological properties of the proposed Substituted p-Phenylenediamines category members are similar and follow a regular pattern as a result of that structural similarity. Therefore, the EPA definition of a chemical category has been met. Further, the availability and results of data for the chemicals that constitute the Substituted p-Phenylenediamines category indicate that no additional testing needs to be conducted for the purposes of the HPV Program.

Introduction

A provision for the use of structure activity relationships (SAR) to reduce potential testing is included under EPA's HPV Program. Specifically, categories may be formed based on structural similarity, through analogy, or through a combination of category and analogy for use with single chemicals. The benefits of using a category approach are numerous and include accelerated release of hazard information to the public (category analysis and testing are proposed to be initiated within the first two years of the HPV Program); reduction in the number of animals used for testing; and an economic savings as a result of a reduced testing program.

The Substituted p-Phenylenediamines that form this category based on structural similarity are:

Alkylated N-PPD
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)

4-Aminodiphenylamine Derivatives
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3)

The category has been arranged into two primary subcategories (Alkylated N-PPD and 4-Aminodiphenylamine Derivatives) for purposes of bridging data to the closest related material. The materials were further arranged in order of molecular weight, so that the smallest material is listed first, and the following materials have increasingly larger molecular weights. Of these, p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4) has been evaluated in the Organization for Economic Co-operation and Development (OECD) Screening Information Data Set (SIDS) program and p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8) is currently in the OECD SIDS evaluation process. Data for these two members of the Substituted p-Phenylenediamines category are included in support of the five category members sponsored in the HPV Program.

The development of this category follows current EPA guidance[1].

Background Information: Manufacturing and Commercial Applications

Manufacturing

Substituted p-Phenylenediamines are manufactured batchwise in high-pressure autoclave reactors using a process known as catalytic reduction. In a typical reaction process, the chemical intermediate 4-Aminodiphenylamine (CAS#101-54-2) is reacted with the appropriate ketone and hydrogen gas in the presence of a precious metal catalyst on carbon to form the product, which is then purified via separation, filtration and azeotropic distillation.

[1] US EPA, Office of Pollution Prevention and Toxics. Development of Chemical Categories, Chemical Right-to-Know Initiative. http://www.epa.gov/opptintr/chemrtk/categuid.htm.

Commercial Applications

In the U.S., Substituted p-Phenylenediamines are used primarily as antidegradants in the production of black or dark-colored rubber, as fuel additives and in monomer distillation processes. They are widely used in the manufacture of tires (sidewall, tread and retread, carcass, belt skim, liner, bead filler/chafer, and base tread), moldings, hoses, belts and gaskets for the automotive industry and in other industrial rubber products such as roofing material that are exposed to the elements. Others are used as fuel additives to prevent air oxidation, and a few find usage as "short-stoppers" or polymerization inhibitors in the process of monomer distillation. Substituted p-Phenylenediamines are powerful antioxidants/antiozonants that greatly extend the useful life of rubber articles and functional fluids by delaying the oxidative aging process. These highly-colored, or "staining" antidegradants also help prevent surface cracking caused by flex fatigue in dynamic applications. Typical usage level for the Substituted p-Phenylenediamines in these industrial applications ranges from 0.5 – 3%.

FDA Status – The Substituted p-Phenylenediamines are not widely used in food contact applications because of their capability to stain and discolor. However, two chemicals in this category have some limited food-contact applications:

Federal Regulation	Application	CAS No.
175.105	Components of Adhesives	68953-84-4
177.2600	Rubber Articles	68953-84-4 and 101-72-4

Shipping/Distribution

Substituted p-Phenylenediamines are shipped extensively throughout the world from manufacturing plants located in North and South America, Eastern and Western Europe, China and Japan. These materials are typically shipped by tank car, tank truck, and barge.

Worker/Consumer Exposure

The rubber and plastics additives industry has a long safety record and sophisticated industrial users handle the materials. Exposure of workers handling substituted p-phenylenediamines category chemicals is likely to be greater in the area of material packaging than in manufacturing. These materials are made as pastilles (pellets), powders, flakes, solids and liquids. Thus, during the transfer operation from the manufacturing process to packaging there is a potential for inhalation exposure (nuisance dust is the primary route of worker exposure) and dermal contact to liquid forms. There should be little, if any, consumer exposure to Substituted p-Phenylenediamines since these materials will be part of finished articles, and as such unavailable for exposure or release under typical conditions of use.

Development of the Substituted p-Phenylenediamines Category

EPA has described a stepwise process for developing categories. These steps include:

- Grouping a series of like chemicals, including the definition of criteria for the group.
- Gathering data on physicochemical properties, environmental fate and effects, and health effects for each member of the category.
- Evaluating the data for adequacy.
- Constructing a matrix of available and unavailable data.
- Determining whether there is a correlation among category members and data gathered.

Definition of the Substituted p-Phenylenediamines Category

As defined by EPA under the HPV Program, a chemical category is "a group of chemicals whose physicochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity." The similarities should be based on a common functional group, common precursors or breakdown products (resulting in structurally similar chemicals) and an incremental and constant change across the category. The goal of developing a chemical category is to use interpolation or extrapolation to assess chemicals rather than conducting additional available testing with specific consideration of animal welfare concerns to minimize the use of animals in the testing of chemicals.

The materials within the Substituted p-Phenylenediamines category, for the purposes of the HPV Program, are defined as phenylenediamines with alkyl, aryl or mixed alkyl-aryl substitutions, as illustrated in Figure 1.

The category referred to as Substituted p-Phenylenediamines is further categorized into two secondary subcategories: Alkylated N-phenylenediamines and 4-Aminodiphenylamine derivatives. The Alkylated N-phenylenediamines materials are structurally similar in that both N groups are alkylated, while the 4-Aminodiphenylamine derivatives materials all contain aryl and alkyl substituted groups. Chemical structures for these materials are illustrated in Figure 2. The very low water solubility, low vapor pressure, slow biodegradation, low bioaccumulation potential, rapid hydrolysis and photodegradation are similar for the Substituted p-Phenylenediamines (see Tables 1 and 3). These highly-colored, staining compounds also have high flash points (see Table 1).

Matrix of SIDS Endpoints

In order to construct a matrix of SIDS endpoints for the members of the Substituted p-Phenylenediamines category, the data on physicochemical properties, environmental fate and effects, and health effects for each member of the category must be collected and evaluated for adequacy. The results of these activities are presented in the tables and text below, providing a matrix of available data for the Substituted p-Phenylenediamines materials.

Correlation within the Substituted p-Phenylenediamines Category

The matrix data patterns for physicochemical properties; environmental fate, ecotoxicity; and health effects have been evaluated for the members of the Substituted p-Phenylenediamines category. A description of the results of this evaluation follows.

Correlation of Physicochemical Properties

The physicochemical properties of the members of the Substituted p-Phenylenediamines category are presented in Table 2. These materials may exist as viscous liquids or solids at room temperature, such that melting point or boiling point data may be relevant for varying members of the category. The similarities in the other physicochemical properties of these materials, which are described below, are explained by similarities in their chemical structure, and provide justification of this group of chemicals as a category within the HPV Challenge Program.

Members of this category have a wide range of melting points and boiling points (varying based on the physical state as a liquid or solid). Six members of the category have very low vapor pressures, as indicated in Table 2. Data for six members of the category clearly indicate a lack of water solubility or negligible water solubility. Partition coefficient data are primarily in the range of 3 to 5.

Bridging to other members of the category or use of EPIWIN modeling will be used to address physicochemical properties data requirements for the purposes of the HPV Program, as illustrated below, and in Table 1.

Alkylated N-Phenylenediamines: Sufficient data exist for the Alkylated N-Phenylenediamines materials for the purposes of the HPV Program.

4-Aminodiphenylamine Derivatives: Physicochemical properties data (boiling point and vapor pressure) for p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8) are provided by EPIWIN modeling. Vapor pressure, boiling point and water solubility data will be bridged from p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) to 1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4). Partition coefficient data for p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) will be bridged to p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3). EPIWIN was used to provide melting point and vapor pressure data for p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3).

Correlation of Environmental Fate

The members of this category are generally found to be not readily biodegradable by CO_2 generation, but photodegradation is rapid, as is hydrolysis. Analytical studies of hydrolysis products indicate that the molecule cleaves at the aromatic carbon-nitrogen bond.

The HPV Challenge Program requires that hydrolysis, photodegradation, biodegradation and environmental transport information be presented for each material or bridged to each member of a category. Adequate biodegradation data exist for several of the materials in this category for the purposes of the HPV Program; bridging will be used to address the remaining biodegradation data requirements as illustrated below. The results presented indicate that these materials are poorly biodegradable, with the exception of p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4) and p-Phenylenediamine, N-(1,3-diemthylbutyl) N'-phenyl-, (793-24-8). Hydrolysis data exists for several members of this group, and gas chromatography identification and quantification of hydrolysis products suggests a common breakdown mechanism exists. Photodegradation studies presented for several

members of this category are adequate for the purposes of the HPV Program; bridging will be used to address the remaining photodegradation data requirements as illustrated below. Finally, fugacity modeling has been conducted on six of the seven members of the category, with consistent results showing partitioning to soil and/or sediment. This finding is consistent with the lack of water solubility and low vapor pressure of these materials. Bridging to other members of the category will address environmental transport data requirements, as illustrated below.

Alkylated N-Phenylenediamines: The hydrolysis data for p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9) will be bridged to p-Phenylenediamine, N,N-di-sec-butyl (101-96-2). Biodegradation and photodegradation data for p-Phenylenediamine, N,N-di-sec-butyl (101-96-2) was modeled using EPIWIN.

4-Aminodiphenylamine Derivatives: Photodegradation, hydrolysis, and environmental transport data will be bridged from p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8) to 1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4). Photodegradation data was modeled using EPIWIN for p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4), p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8), p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4 and p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3).

Biodegradation data for p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3) was modeled using EPIWIN.

Correlation of Ecotoxicity

The HPV Challenge Program requires that an acute aquatic ecotoxicity test in fish, invertebrates, and algae be performed or bridged to each member of a category. Existing data (Table 4) indicate that six members of the Substituted p-Phenylenediamines category have low water solubility. The low water solubility suggests that the acute aquatic toxicity of these materials should be low due to limited bioavailability to aquatic organisms. However, the Substituted p-Phenylenediamines, in general, are very toxic to aquatic organisms. Additional testing is not necessary for these materials for the purposes of the HPV Program.

Alkylated N-Phenylenediamines: Results of acute aquatic toxicity studies show p-Phenylenediamine, N, N-bis(1,4-dimethylpentyl) (3081-14-9) is harmful to algae, and very toxic to fish and Daphnia. P-Phenylenediamine, N, N-di-sec-butyl (101-96-2) was very toxic to fish and toxic to Daphnia in acute aquatic studies. The algal growth inhibition data for p-Phenylenediamine, N, N-bis(1,4-dimethylpentyl) (3081-14-9) will be bridged to p-Phenylenediamine, N, N-di-sec-butyl (101-96-2).

4-Aminodiphenylamine Derivatives: Aquatic toxicity data exist for four of the five members of this subcategory. The results of aquatic toxicity testing of these materials indicate they are toxic to very toxic to fish, Daphnia, and algae in acute studies.

The acute aquatic toxicity data for p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) will be bridged p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3).

Correlation of Health Effects

Acute Mammalian Toxicity

Acute oral and dermal toxicity data for the Substituted p-Phenylenediamines category are summarized in Table 5. The two materials in the Alkylated N-Phenylenediamines subcategory of the Substituted p-Phenylenediamines show a moderate order of acute oral toxicity. The second subcategory, the 4-Aminodiphenylamine derivatives, all exhibit a very low order of toxicity, with LD_{50} values greater than the limit test of 2000 mg/kg with the exception of p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4), with an oral LD_{50} of 900 mg/kg. Acute dermal toxicity data for all members of the Substituted p-Phenylenediamines category demonstrate a very low order of toxicity with the dermal LD_{50} values greater than the limit test of 2000 mg/kg.

Adequate acute toxicity studies have been conducted for the Substituted p-Phenylenediamines category. These studies involved at least two routes of exposure (oral and dermal); and evaluated the toxicity of all the members of the category. The data demonstrate a moderate to very low order of acute toxicity. The trend in acute oral toxicity follows the molecular weight of the materials. That is, there is a general trend toward decreasing acute oral toxicity with increasing molecular weight. The similarity in the order of toxicity for these materials is consistent with their similar chemical structure and physicochemical properties and supports the scientific justification of these materials as a category within the HPV Challenge Program.

The HPV Challenge Program requires that either an acute test be performed or bridged to each member of a category. Adequate acute oral and dermal toxicity tests exist for the Substituted N-Phenylenediamines for the purposes of the HPV Program.

Mutagenicity

A summary of the mutagenicity information for the Substituted p-Phenylenediamines category is presented in Table 6. The weight of evidence for the members of this category indicates these materials are not mutagenic.

Adequate bacterial mutagenicity tests have been conducted for all seven of the Substituted N-Phenylene-diamines category to satisfy HPV Challenge requirements. Similarly, adequate *in vitro* chromosome aberration tests or *in vivo* micronucleus tests have been conducted for five of the seven materials in the Substituted N-Phenylenediamines category: additional *in vitro* or *in vivo* mammalian mutagenicity studies are available as supporting information.. Bridging will be used to fill the remaining data requirement.

<u>Bacterial Gene Mutation Assay</u>

With one exception, mutagenicity was not exhibited by any of the materials in the Substituted p-Phenylenediamines category in the bacterial mutagenicity tests with or without metabolic activation. The single exception was a positive response with 1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4).

In vivo Chromosomal Aberration Assays (Mammalian Micronucleus Test)
Three of the seven Substituted p-Phenylenediamine materials have been adequately tested in an *in vivo* chromosomal aberration assay for HPV Challenge requirements. The results were negative for clastogenicity.

In vitro Chromosomal Aberration Assay
Six of the seven Substituted p-Phenylenediamine materials have been adequately tested in an *in vitro* chromosomal aberration assay using Chinese hamster ovary cells to satisfy Program requirements. The results of these studies, performed with and without metabolic activation of the test material, were negative for clastogenicity with the exception of p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4).

The Substituted p-Phenylenediamines category has been adequately tested for mutagenicity in tests for gene mutations and chromosomal aberrations for purposes of meeting HPV Challenge requirements. The assays included point mutations in bacterial cells, *in vitro* chromosomal aberrations in mammalian cells, and *in vivo* chromosomal aberrations. The data consistently demonstrate no evidence of genotoxicity for this category of materials. 1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4) was positive in the bacterial mutagenicity test, but was negative in both *in vitro* and *in vivo* mammalian mutagenicity studies. p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) was positive for clastogenicity in the *in vitro* chromosome aberration test, but was negative in the *in vivo* mouse micronucleus test. This suggests that all members of the category lack genotoxicity because of their similarity in chemical structures and physicochemical properties. The similarity of results for genotoxicity supports treatment of these materials as a chemical category within the HPV Challenge Program.

The HPV Challenge Program requires that a gene mutation and a chromosomal aberration test be performed or bridged to each member of a category. Bridging will be used to fill the remaining data requirements.

Alkylated N-Phenylenediamines: Sufficient data exist for the Alkylated N-Phenylenediamines materials for the purposes of the HPV Program.

4-Aminodiphenylamine Derivatives: Data from *in vivo* mutagenicity testing with p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8) will be bridged to p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4). Mutagenicity test data from p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) will be bridged to p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3).

By bridging these data, the category has been evaluated adequately for genotoxicity for the purposes of the HPV Program, and no additional testing is proposed**.**

Repeat Dose Toxicity
A summary of the repeat dose toxicity data for the Substituted p-Phenylenediamines category is presented in Table 7.

Alkylated N-Phenylenediamines: Adequate repeat dose studies are available for both the Alkylated N-Phenylenediamines materials for the purposes of the HPV Program. P-Phenylenediamine, N, N-bis (1,4-dimethylpentyl) (3081-14-9) was given in the diet to rats at levels of 0, 100, 300, 500, 1000, or 2000 ppm (5/sex/group) for four weeks. Males at 300 ppm and above and females at 1000 ppm and above showed a reduced body weight gain. Alterations in hematology and clinical chemistry parameters were noted at the two highest dose levels. The No Observed Effect Level (NOEL) for males and females was 100 and 300 ppm, respectively. 100 male and female rats (10/sex/dose level) were dosed with p-Phenylenediamine, N, N-di-sec-butyl (101-96-2) in corn oil vehicle at 0, 10, 25, 50, or 100 mg/kg for a period of 28 days. Because the results of this study demonstrated hepatic effects in both sexes and at all treatment levels, a NOEL could not be established.

4-Aminodiphenylamine Derivatives: Adequate repeat dose studies are available for four of the five 4-Aminodiphenylamine Derivatives materials for the purposes of the HPV Program.

Subchronic studies have been conducted with p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4). When administered to rats in the diet at levels of 0, 500, 1000, 1750 and 2500 ppm for four weeks, decreases in body weight gains, hematological effects, elevations in total serum protein and increased lever and spleen weight were noted at 1000 ppm and above. The NOEL was identified as 500 ppm. In a 90-day study, p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4) was administered to rats in the diet at levels of 0, 180, 360 or 720 ppm. Lower body weight gains were observed in high-dose males; increased absolute and relative liver weights were noted in mid- and high-dose males and all treated females. Increased spleen and kidney weights were observed in high-dose females, and mild anemia was noted in mid- and high-dose animals. There were no treatment related gross or histopathological changes noted in any group. A NOEL for organ weight changes was not established for females, while a NOEL for males was 180 ppm.

Dietary administration of p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) at 0, 500, 750, 1500 or 3000 ppm to rats for one month resulted in reduced food consumption and decreased weight gain at the three highest doses in both sexes. No gross pathology or other signs of toxicity were noted. The NOEL was identified as 500 ppm in the diet.

Dietary administration of 1,4-Benzenediamine, -mixed Ph and toyl derivatives (68953-84-4) at concentrations of 0, 120, 470 and 1900 ppm (0, 7.5, 30 and 120 mg/kg/day) to rats for 28 days resulted in body weight decreases in high dose female rats and decreased food consumption in high-dose males and mid- and high-dose females. Hematological changes (high dose), liver and kidney weight increases (high-dose male and female, mid-dose females). The No Observed Adverse Effects Level (NOAEL) for this study was established at 7.5 mg/kg. A 21-day gavage range-finding study was also conducted with rats with this material at doses of 0, 0.1, 0.3, 1 and 3 g/kg/day. Lethality was observed at 1 and 3 g/kg/day. Body weight gain loss, liver weight increase and hepatocellular labeling index increase were noted at 0.3 and/or 0.1 g/kg/day.

Santoflex 13 (p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)) was administered in feed to groups of 6 week old male and female rats at 0, 250, 1000 or 2500 ppm. Analyses via GC verified feeding levels of 0, 230, 950 and 2300 ppm. All animals survived the length of the study. Signs of toxicity during the study were limited to reduced feed consumption/body weight gain in the high-dose males and

females and mid-level males. Anemia, lymphocytopenia and thrombocytosis were present in males and females, primarily at the two highest dose levels. Increases in total bilirubin in males, and total protein, albumin, globulin, calcium and/or cholesterol in both sexes were noted in high and some mid-dose level animals. Increased liver weights were observed at the two highest dose levels. There were no gross or microscopic lesions attributed to consumption of the test material. Females at low dose levels exhibited mild anemia at the interim sampling period, but all recovered by the end of the study. Therefore, the NOEL was considered to be 250 ppm.

Repeat dose data from p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4) will be bridged to p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3).

By bridging these data, the category has been evaluated adequately for genotoxicity for the purposes of the HPV Program, and therefore, no additional testing is proposed**.**

Reproductive and Developmental Toxicity A summary of the reproductive and developmental toxicity data for the Substituted p-Phenylenediamines category is presented in Table 7.

Alkylated N-Phenylenediamines: Adequate reproductive toxicity studies are available for the purposes of the HPV Program for one of the two Alkylated N-Phenylenediamines materials. P-Phenylenediamine, N, N-bis (1,4-dimethylpentyl) (3081-14-9) was not embryotoxic, fetotoxic or teratogenic when administered by gavage at doses of 0, 25, 75 or 150 mg/kg/day to pregnant rats on gestation days 6-15. Administration of CAS No. 3081-14-9 at dietary concentrations of 0, 30, 100 or 300 ppm to male and female rats for three successive generations produced no adverse effects on mating or fertility indices. Reduced survival of offspring was observed in mid- and high-dose groups; however, evidence of parental toxicity was also present as indicated by reduced body weight gains of mid- and high-dose groups. The NOEL was 30 ppm. The developmental and reproductive studies with p-Phenylenediamine, N, N-bis (1,4-dimethylpentyl) (3081-14-9 will be bridged to p-Phenylenediamine, N, N-di-sec-butyl (101-96-2).

4-Aminodiphenylamine Derivatives: Adequate reproductive and developmental toxicity studies are available for three of the five 4-Aminodiphenylamine Derivatives materials for the purposes of the HPV Program.

p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4) was administered to rats by gavage at dose levels of 0, 12.5, 62.5 or 125 mg/kg/day for gestation days 6-15. The NOEL for maternal toxicity was determined to be 62.5 mg/kg. There were significant skeletal effects at 125 mg/kg and the NOEL for teratogenicity was established at 62.5 mg/kg.

1,4-Benzenediamine, N, N'-mixed Ph and toyl derivatives (68953-84-4) was administered in feed at 0, 120, 400 or 1500 ppm to rats in a two-generation reproductive toxicity study. Dystocia (potentially leading to prolonged gestation and increased perinatal deaths, decreased live births and increased pup weights), and polycystic lesions were observed at all dose levels; a NOAEL was not established in this study. A developmental study was also conducted with 1,4-Benzenediamine, N, N'-mixed Ph and toyl derivatives (68953-84-4) in rats. The test article was administered by gavage at dose levels of 0, 20, 70 and 200 mg/kg/day for gestation days 6-15. The test article produced minimal effects (body weight) to

maternal rats at 200 mg/kg during pregnancy; the NOAEL for maternal toxicity was established at 70 mg/kg/day. There were no birth defects observed in fetal animals and the NOAEL for teratogenicity/developmental effects was established at 200 mg/kg/day.

A reproductive oral gavage study was conducted in rats with p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8); no reproductive effects were observed at the highest concentration tested (1000 ppm). In a rat gavage developmental study, the test article was administered by gavage at dose levels of 0, 50, 100 and 250 mg/kg/day for gestation days 6-15. A NOAEL (teratogenicity /developmental effects) greater than 250 mg/kg/day was determined. The NOEL for maternal toxicity was established at 50 mg/kg/day.

Data from these three studies materials will be bridged to p-Phenylenediamine, N- (1,4-dimethylpentyl) N'-phenyl (3081-01-4) and p-Phenylenediamine, N, (1-methylheptyl)-N'-phenyl, (15233-47-3).

Test Plan

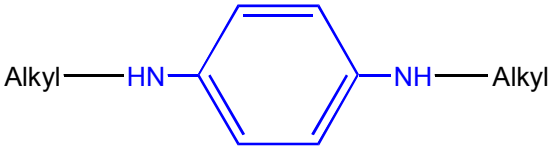
Table 8 provides the category test plan for the Substituted p-Phenylenediamines. All HPV endpoint requirements are fulfilled by existing adequate data, calculated data, or by bridging data based on SAR and the category approach. The chemicals that constitute the Substituted p-Phenylenediamines category require no additional testing for the purposes of the HPV Program

FIGURES

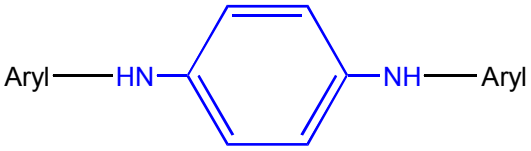
Figure 1. Structural Definition

Phenylenediamine with various aryl or alkyl substitutions in the para position:

Alkyl-N-Phenyl-N-Alkyl (all Alkyl)
Aryl-N-Phenyl-N-Aryl (All Aryl)
Alkyl-N-Phenyl-N-Aryl (Mixed Alkyl-Aryl)



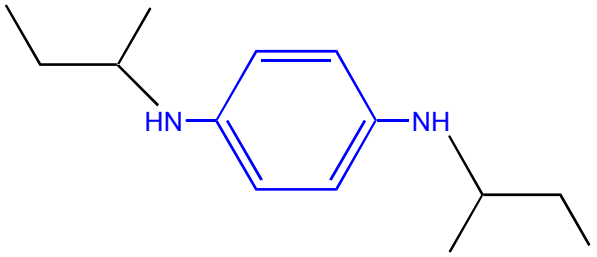
Alkyl-Alkyl Substitutions



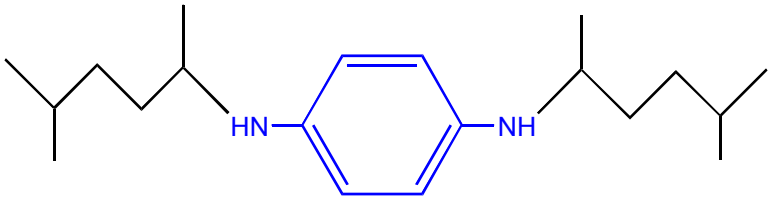
Aryl-Aryl Substitutions

Mixed Alkyl-Aryl Substitutions

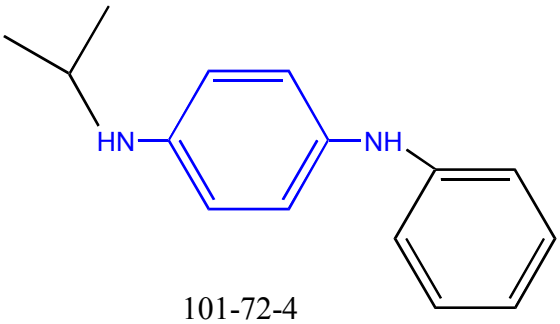
Figure 2. Chemical Structures



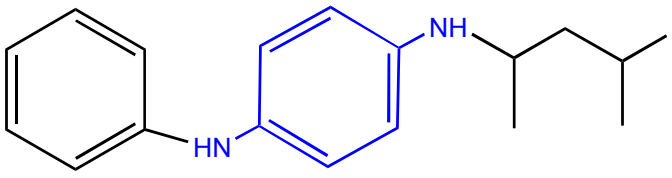
101-96-2
Alkyl-Alkyl



3081-14-9
Alkyl-Alkyl



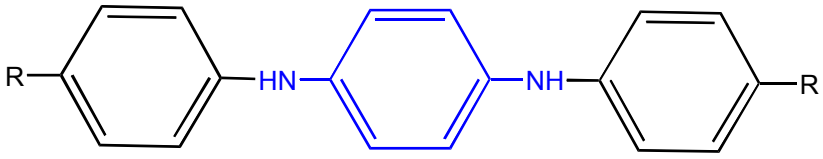
101-72-4
Alkyl-Aryl



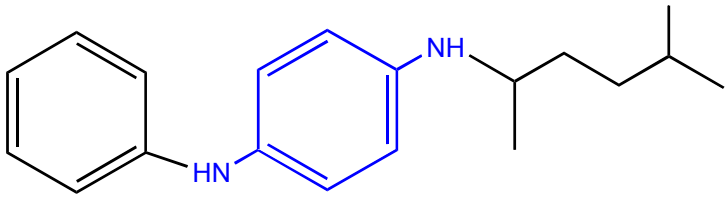
793-24-8
Alkyl-Aryl

Figure 2. Chemical Structures (Continued)

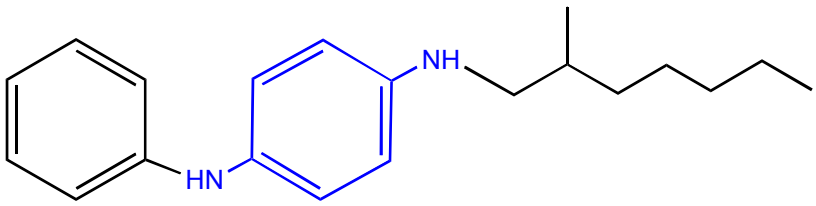
R = H or CH3



68953-84-4
Aryl-Aryl (Mixed)



3081-01-4
Alkyl-Aryl



15233-47-3
Alkyl-Aryl

TABLES

Table 1. Justification of the Substituted p-Phenylenediamines Category using Flash Point, Vapor Pressure, Water Solubility and Biodegradation

Name (CAS No.)/ Molecular weight	Flash Point (^{o}F)	Vapor Pressure (mm Hg @ 20^{o}C)	Water Solubility	Bio-degradability
Alkylated N-Phenylenediamines				
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)/ 220.4	290	85.3 @ 33C	Insoluble	Not readily biodegradable
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)/ 304	182	1.1 @ 25C	Very Slight	Not readily biodegradable
4-Aminodiphenylamine derivatives				
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)/ 226.4	>200 C	3.4E-5 @ 90C	Insoluble	Readily biodegradable
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)/ 268.5	400	4.93E-6 @ 25C (EPIWIN)	Insoluble	Readily biodegradable
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)/ 274	450	Not determined	Not determined	Not readily biodegradable
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)/ 282	420	1.25E-10 @25C	Insoluble	Not readily biodegradable
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3)/ 296	Not determined	4.99E-7 @ 25C (EPIWIN)	Insoluble	Not readily biodegradable

Table 2. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members
Physicochemical Properties

Name (CAS No.)	Melting Point (^{o}C)	Vapor Pressure (mm Hg @ 20^{o}C)	Boiling Point (^{o}C)	Partition Coefficient	Water Solubility
Alkylated N-Phenylenediamines					
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	18	85.3 @ 38 C	98 @ 26.6hPa	3.50	<1 mg/ml @ 20C
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	-36	<1.1E-6 @ 25C	183.5 @ 1mm Hg	5.34	21 ppm @ pH5; 0.8 ppm @ pH 9
4-Aminodiphenylamine derivatives					
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	75-80	3.4E-3 @90C	161	3.28	15 ppm @25C
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	45	4.93E-6 @25C (EPIWIN)	369.67 (EPIWIN)	4.7	1 ppm @ 23C
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	90-105	Not determined	Not determined	3.4-4.3	Not determined
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	32	1.25E-10 @ 25C	231 @3.5 mmHg	5.17	0.67g/l @ 25C
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3)	145.77 (EPIWIN)	4.99E-7 @ 25C (EPIWIN)	431	Not determined	Insoluble

Table 3. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members Environmental Fate

Name (CAS No.)	Hydrolysis	Photo-degradation (t1/2 in hours)	Bio-degradation	Environmental Transport
Alkylated N-Phenylenediamines				
p-Phenylenediamine, N, N-di-sec-butyl (101-96-2)	Not determined	1.095 (EPIWIN)	Not readily biodegradable (EPIWIN)	Primarily to soil
p-Phenylenediamine, N, N-bis(1,4-dimethylpentyl) (3081-14-9)	97%@pH7 after 24 hr	2	50% after 35 days	Primarily to sediment
4-Aminodiphenylamine derivatives				
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	99%@pH7 after 24 hr	0.588 (EPIWIN)	98% after 22 hours	Primarily to soil
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	93%@pH7 after 24 hr	0.567 (EPIWIN)	50 % after 2.9 hours	Primarily to soil
1,4-Benzenediamine, N, N'-mixed Ph and toyl derivatives (68953-84-4)	Not determined	Not determined	0.64% after 28 days	Not determined
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	96%@pH7 after 24 hr	0.563 (EPIWIN)	0% @ 35days	Primarily to soil
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	Not determined	0.56 (EPIWIN)	Not readily biodegradable (EPIWIN)	Primarily to soil and sediment

Table 4. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members Ecotoxicity

Name (CAS No.)	Acute Fish 96-hour LC50 (mg/l)	Acute Invertebrate 48-hour EC50 (mg/l)	Algal growth inhibition 96-hour EC50 (mg/l)
Alkylated N-Phenylenediamines			
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	0.13	1.4	Not determined
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	0.28	0.37	52
4-Aminodiphenylamine derivatives			
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	0.34	1.1	0.5 (cell growth)
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	0.14-0.4	0.82	0.6
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	0.48	1.8	(72-hour EC50) 0.018 (biomass); >0.079 (growth rate)
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	0.3-1.1	0.2	0.7
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	Not determined	Not determined	Not determined

Table 5. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members
Acute Toxicity

Name (CAS No.)	Acute Oral (mg/kg)	Acute Dermal (mg/kg)
Alkylated N-Phenylenediamines		
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	271	2806
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	730	>3160
4-Aminodiphenylamine derivatives		
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	900	>7940
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	>5000	>7940
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	>2000	>2000
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	>2000	>5010
p-Phenylenediamine, N,(1-methylheptyl)-N'-phenyl, (15233-47-3)	4300	>2000

Table 6. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members
Genotoxicity

Name (CAS No.)	Genotoxicity (*in vitro* - bacterial)	Genotoxicity (*in vitro* - mammalian)	Genotoxicity (*in vivo*)
Alkylated N-Phenylenediamines			
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	Negative	Negative	Not determined
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	Negative	Negative	Not determined
4-Aminodiphenylamine derivatives			
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	Negative	Negative	Not determined
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	Negative	Negative	Negative
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	Positive	Negative	Negative
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	Negative	Weak Positive; Supporting data Negative	Negative
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	Negative	Not determined	Not determined

Table 7. Matrix of Available and Adequate Data on Substituted p-Phenylenediamines Category Members
Health Effects

Name (CAS No.)	Repeat Dose	Reproductive	Developmental
Alkylated N-Phenylenediamines			
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	28-Day oral gavage with rats. NOEL< 10 mg/kg/day	Not determined	Not determined
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	30 day feeding study with rats. NOEL (males) 100 pm; (females) 300 ppm	Three generation rat oral feeding study; NOEL(parental, F1 and F2 offspring) = 30 ppm	Rat gavage: NOEL (teratogenicity) = >150 mg/kg/day; (maternal) = 25 mg/kg/day
4-Aminodiphenylamine derivatives			
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	90-day feeding study with rats. NOEL (males) 180 ppm; NOEL not established (females)	Not determined	Rat gavage: NOEL (teratogenicity) = 62.5, (maternal) 62.5 mg/kg/day
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	90-day oral rat-NOAEL = 250 ppm in feed	Rat gavage – NOEL (parental) >1000 ppm; (F1 offspring) >1000 ppm	Rat gavage: NOAEL (teratogenicity /developmental effects) = 250 mg/kg/day; NOEL (maternal) = 50 mg/kg/day
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	28-day rat oral NOAEL = 7.5 mg/kg	Two generation rat oral feeding study – NOEL not identified	Rat gavage: NOAEL (teratogenicity /developmental effects) $\leq$ 200 mg/kg/day, NOAEL (maternal toxicity 70 mg/kg/day)
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	1 month feeding study with rats – NOEL = 500 ppm in diet	Not determined	Not determined
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	Not determined	Not determined	Not determined

Table 8

Substituted p-Phenylenediamines Category Test Plan

CAS Nos. 101-96-2, 3081-14-9, 101-72-4, 793-24-8, 3081-01-4, 15233-47-3, and 68953-84-4
Rubber and Plastic Additives Panel of the American Chemistry Council
July 2003

	Physical-Chemical				
CHEMICAL	**Melting Point**	**Boiling Point**	**Vapor Pressure**	**Partition Coefficient**	**Water Solubility**
Alkylated N-Phenylenediamines					
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	A	A	A	A	A
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	A	A	A	A	A
4-Aminodiphenylamine derivatives					
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	A	A	A	A	A
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	A	Calc	Calc	A	A
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	A	R	R	A	R
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	A	A	A	A	A
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	R	A	Calc	R	A

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

Table 8 (continued)

	Environmental Fate			
CHEMICAL	**Photo-degradation**	**Hydrolysis**	**Environmental Transport**	**Biodegradation**
Alkylated N-Phenylenediamines				
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	Calc	R	Calc	Calc
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	A	A	Calc	A
4-Aminodiphenylamine derivatives				
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	Calc	A	Calc	A
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	Calc	A	Calc	A
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	R	R	R	A
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	Calc	A	Calc	A
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	Calc	R	Calc	Calc

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

Table 8 (continued)

	Ecotoxicity		
CHEMICAL	**Acute Toxicity to Fish**	**Acute Toxicity to Aquatic Plants (e.g., Algae)**	**Acute Toxicity to Aquatic Invertebrates (e.g., Daphnia)**
Alkylated N-Phenylenediamines			
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	A	R	A
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	A	A	A
4-Aminodiphenylamine derivatives			
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	A	A	A
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	A	A	A
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	A	A	A
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	A	A	A
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	R	R	R

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

Table 8 (continued)

	Mammalian Toxicity						
CHEMICAL	**Acute Toxicity**	**Genetic Toxicity *In Vitro* (bacterial)**	**Genetic Toxicity *In Vitro* (mammalian)**	**Genetic Toxicity *In Vivo***	**Repeat Dose Toxicity**	**Repro-ductive Toxicity**	**Develop-mental Toxicity**
Alkylated N-Phenylenediamines							
p-Phenylenediamine, N,N-di-sec-butyl (101-96-2)	A	A	A	NR	A	R	R
p-Phenylenediamine, N,N-bis(1,4-dimethylpentyl) (3081-14-9)	A	A	A	NR	A	A	A
4-Aminodiphenylamine derivatives							
p-Phenylenediamine, N-Isopropyl-N'-phenyl-, (101-72-4)	A	A	A	R	A	R	A
p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (793-24-8)	A	A	A	A	A	A	A
1,4-Benzenediamine, N,N'-mixed Ph and toyl derivatives (68953-84-4)	A	A	A	A	A	A	A
p-Phenylenediamine, N-(1,4-dimethylpentyl) N'-phenyl (3081-01-4)	A	A	R	A	A	R	R
p-Phenylenediamine, N,(1methylheptyl)-N'-phenyl, (15233-47-3)	A	A	R	R	R	R	R

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

101-96-2
1,4-Benzenediamine, N, N'-bis(1-methylpropyl)-

Molecular Weight: **220.36**
Molecular Formula: **C14-H24-N2**

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance: Organic
B. Physical State: Dark reddish-brown liquid
C. Purity: 96-99 % Typical for Commercial Products

1.2 SYNONYMS

Santoflex® 44PD
Santoflex® 44
Kerobit® BPD
UOP 5®
Tenamine® 2
Topanol® M
Antioxidant PDA®
Antioxidant 22®
N,N'-di-sec-butyl-p-phenylenediamine

1.3 IMPURITIES Various low-level isomers

1.4 ADDITIVES None

2. PHYSICAL-CHEMICAL DATA

***2.1 MELTING POINT**

Value:	15.9°C (onset)
Decomposition:	No
Sublimation:	No
Method:	Instrumental – Differential Scanning Calorimeter, 2002
GLP:	Yes
Remarks:	Sample had a purity of 99.1%. Product is known to super-cool. Glass transition temperature <0°C. Exotherm at –30°C
Reference:	Flexsys Analytical Research Report #2002.043, 2002
Reliability:	(1) Valid without restriction

Value:	17.8°C (crystallizing point)
Decomposition:	No
Sublimation:	No
Method:	FF88.2-1 Crystallizing Point of Organic Compounds, 1997
GLP:	Yes
Remarks:	Sample had a purity of >97%. Product is known to super-cool.
Reference:	Flexsys Standard Methods of Analysis
Reliability:	(1) Valid without restriction

***2.2 BOILING POINT**

Value: 225°C

Pressure:	1013 hPa
Decomposition:	No
Method:	Instrumental – Differential Scanning Calorimeter, 2002
GLP:	Yes
Remarks:	Thermal stability investigation via DSC showed an endotherm starting at 225°C that was attributed to boiling.
Reference:	Flexsys Analytical Research Report #2002.14, 2002
Reliability:	(1) Valid without restriction

Value:	171°C @ 133.3 hPa 138°C @ 26.6 hPa 128°C @ 1.3 hPa
Decomposition:	No
Method:	No data
GLP:	No data
Remarks:	Boiling point at reduced pressures
Reference:	Monsanto Report # MAK004, January, 1983
Reliability:	(2) Valid with restrictions – no method details

†2.3 DENSITY (relative density)

Type:	Density
Value:	0.94
Temperature:	15.5°C
Method:	FF97.4/ASTM D891-94, 1997
GLP:	Yes
Remarks:	Specific Gravity of Liquids by Hydrometers. Hydrometers must meet ASTM E100 specifications
Reference:	Flexsys Standard Methods of Analysis, April 14, 1997
Reliability:	(1) Valid without restriction

*2.4 VAPOUR PRESSURE

Value:	13.33 hPa
Temperature:	170°C
Method:	No data
GLP:	No data
Remarks:	Equivalent to 10 mm Hg
Reference:	Monsanto Toxicology Profile of Santoflex 44 Antiozonant, 1993
Reliability:	(2) Valid with restrictions – no method detail

*2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

Log Pow:	3.50
Temperature:	Not determined
Method:	calculated SRC LogKow (KowWin) Program 1995
GLP:	No
Remarks:	Estimation based on melting point of 15.9°C and boiling point of 225°C
Reference:	Meylan, W.M. and. P.H. Howard, 1995 J. Pharm. Sci. 84: 83-92
Reliability:	(2) Valid with restrictions – modelling data

***2.6 WATER SOLUBILITY**

A. Solubility

B.

Value:	<1 g/l
Temperature:	20°C
Description:	Of very low solubility
Method:	Not determined
GLP:	no data
Remarks:	Radian Research
Reference:	NTP Chemical Repository, 2001
Reliability:	(4) Not assignable. Data from a secondary literature source

Value:	95.75 mg/l
Temperature:	25°C
Description:	low solubility
Method:	WSKOW v1.40
GLP:	No
Remarks:	Estimation based on melting point of 15.9°C and boiling point of 225°C
Reference:	EPIWIN/WSKOW v1.40
Reliability:	(2) Valid with restrictions – modelling data

2.7 FLASH POINT (liquids)

Value:	143°C
Type:	Tag Closed Cup (TCC)
Method:	ASTM D 56-96, 1996
Reference:	ASTM Standard Test Method for Flash Point by Tag Closed Tester, 1996
Reliability:	(1) Valid without restrictions

2.8 AUTOFLAMMABILITY (liquids)

Value:	329°C
Type:	Tag Open Cup (TOC)
Method:	ASTM D 1310
Reference:	NFPA, Fire Protection Guide to Hazardous Materials, 1997
Reliability:	(2) Valid with restrictions – reference volume source

B. pH Value, pKa Value

2.11 OXIDISING PROPERTIES

†2.12 OXIDATION: REDUCTION POTENTIAL

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

B. Other data – Henry's Law Constant

Results:	3.058E-004 atm-m3/mole

Remarks: Calculated at 25°C
Reference: EPIWIN/HENRYWIN v3.10
Reliability: (2) Valid with restrictions – modelling data

3. ENVIRONMENTAL FATE AND PATHWAYS

*3.1.1 PHOTODEGRADATION

Type: Air
Indirect Photolysis:
Type of sensitizer: OH
Concentration of sensitizer: 156000 molecule/m3
Rate constant (radical): 117.2377 E-12 cm^3/molecule-sec
Degradation: 50% after 1.095 hours
Method: calculated
AOP Program v1.89, 1999
GLP: No
Test substance: Other (calculated)
Reference: EPIWIN/AopWin v1.89
Reliability: (2) Valid with restrictions – accepted calculation method

*3.1.2 STABILITY IN WATER

*3.2 MONITORING DATA (ENVIRONMENTAL)

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION

*3.3.1 TRANSPORT

Type: Volatility
Media: Water
Method: Estimation Method, 1990
Results: Volatilization half-life from model river: 4.883E+004 hours
Volatilization half-life from model lake: 5.328E+005 hours
Volatilization Constant from water: 1.78E-008 atm-m3/mole
Remarks: Model river = 1 m deep flowing at 1 m/sec and wind velocity of 3 m/sec.
Model lake = 1 m deep flowing at 0.05 m/sec and wind velocity of 0.5 m/sec.
Reference: Handbook of Chemical Property Estimation Methods, 1990
Reliability: (2) Valid with restrictions – modelling data

*3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota-sediment-soil-water
Method: Fugacity level III
EPIWIN v3.10
Results:

	Mass Amount (%)	Half-life (hrs)	Emissions (kg/hr)
Air	0.0952	2.19	1000
Water	26.1	900.00	1000
Soil	72.6	900.00	1000

	Sediment	1.24	3.6e+003	0
	Persistence time estimated at 750 hours			
Remarks:	Calculations based on user input values of Log Kow of 3.50 and melting point of 15.9C			
Reference:	EPISUITE/EPIWIN v3.10			
Reliability:	(2) Valid with restrictions – modelling data			

***3.5 BIODEGRADATION**

***3.7 BIOACCUMULATION**

Species:	Other
BCF:	99.42
Method:	BCFWIN v2.14
GLP:	No
Remarks:	Calculation using measured Log Pow = 3.50
Reference:	EPIWIN/BCFWIN v2.14
Reliability:	(2) Valid with restrictions – modelling data

4. ECOTOXICITY

***4.1 ACUTE/PROLONGED TOXICITY TO FISH**

Type of test:	static Closed system
Species:	Salmo gairdneri (Rainbow Trout)
Exposure period:	96 Hours
Results:	LC_{50} (24h) = >0.18 mg/l LC_{50} (48h) = 0.14 mg/l LC_{50} (96h) = 0.13 mg/l NOEC = 0.056 mg/l LOEC = 0.10 mg/l
Analytical monitoring:	No
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >97%
Remarks:	Test fish were obtained from Spring Creek Hatchery in Lewistown, Montana. Test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations was maintained during the holding period, during which time the fish were fed a standard diet of commercial fish food until 48 hours prior to testing, when feeding was stopped. A 96-hour range-finding test preceded the definitive study. Test fish used had a mean weight of 0.87 g and a mean standard length of 39 mm. The test was conducted in 5-gallon glass vessels containing 15 liters of ABC well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 ppm and pH 8.2. Hardness was 255 ppm and alkalinity, 368 ppm. The test vessels were kept in a water bath at 12°C. Test fish were acclimated to the dilution water and test temperature, and held without food for 48 hours prior to testing. Nanograde Acetone was used to prepare the test solutions and as the solvent control (1.0 ml).

Concentrations tested were 0, 0.018, 0.032, 0.056, 0.10 and 0.18 mg/l. Fish were placed in the testing vessels within 20 minutes of the addition of the test material aliquots. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen values (6.4-8.8 mg/l, 59-81% saturation) and pH ranges (7.9-8.3) were monitored during the testing and remained within acceptable limits. As a quality check, test fish were challenged with Antimycin A. The estimated 96Hr LC50 and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.

Reference: Monsanto AB-83X-036, Analytical Bio-Chemistry Labs, 1983

Reliability: (1) Valid without restriction

Type of test: static
Closed system

Species: Lepomis machrochirus (Bluegill Sunfish)

Exposure period: 96 Hours

Results: $LC_{50\ (24h)}$ = 0.19 mg/l
$LC_{50\ (48h)}$ = 0.18 mg/l
$LC_{50\ (96h)}$ = 0.18 mg/l
NOEC = 0.10 mg/l
LOEC = 0.18 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: >97%

Remarks: Test fish were obtained from Osage Catfisheries in Osage Beach, Missouri. Test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations was maintained during the holding period, during which time the fish were fed a standard diet of commercial fish food until 48 hours prior to testing, when feeding was stopped. A 96-hour range-finding test preceded the definitive study. Test fish used had a mean weight of 0.64 g and a mean standard length of 29.6 mm. The test was conducted in 5-gallon glass vessels containing 15 liters of ABC well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 8.8 mg/l, hardness 255 ppm, alkalinity 368 ppm, and pH 8.1. The test vessels were kept in a water bath at 22°C. Test fish were acclimated to the dilution water and test temperature, and held without food for 48 hours prior to testing. Nanograde Acetone was used to prepare the test solutions and as the solvent control. Concentrations tested were 0, 0.032, 0.056, 0.10, 0.18 and 0.32 mg/l. Fish were placed in the testing vessels within 20 minutes of the addition of the test material aliquots. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen values (2.1-8.8 mg/l, 24-

100% saturation) and pH ranges (7.9-8.1) were monitored during the testing. The low dissolved oxygen readings were made after 96 hours of exposure. Since no significant mortality occurred after 24 hours, the effect on the study results was not significant. As a quality check, test fish were challenged with Antimycin A. The estimated 96Hr LC50 and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.

Reference: Monsanto AB-83X-035, Analytical Bio-Chemistry Labs, 1983

Reliability: (1) Valid without restriction

Type of test: static
Closed system

Species: Pimephales promelas (Fathead Minnows)

Exposure period: 96 Hours

Results: LC_{50} (24h) = 0.13 mg/l
LC_{50} (48h) = 0.13 mg/l
LC_{50} (96h) = 0.13 mg/l
NOEC = 0.10 mg/l
LOEC = 0.18 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: >97%

Remarks: Test fish were obtained from an ABC Laboratories in-house culture. Test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations was maintained during the holding period, during which time the fish were fed a standard diet of commercial fish food until 48 hours prior to testing, when feeding was stopped. Test fish had a mean weight of 0.20 g and a mean standard length of 24 mm. The test was conducted in 5-gallon glass vessels containing 15 liters of laboratory well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 mg/l, hardness (CaCO3) of 255 ppm, alkalinity of 368 ppm, and pH 8.2. The test vessels were kept in a water bath at 22°C.

Test fish were acclimated to the dilution water and test temperature, and held without food for 48 hours prior to testing. Nanograde Acetone was used to prepare the test solutions and as the solvent control (1.0 ml). Test concentrations were 0, 0.056, 0.10, 0.18, 0.32 and 0.56 mg/l for the test compound. Fish were placed in the testing vessels within 20 minutes of the addition of the test material aliquots. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen values and pH ranges were monitored during the testing and remained within acceptable limits of 107-68% saturation (9.4-6.0 mg/l) for dissolved oxygen and pH value (8.3-8.2) consistent

with control. The ammonia concentration was below the toxic limit. Water hardness (CaCO3) was 255 ppm. As a quality check, test fish were challenged with Antimycin A. The estimated 96Hr LC50 and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.

Reference: Monsanto AB-84X-021, Analytical Bio-Chemistry Labs, 1983

Reliability: (1) Valid without restriction

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test: static
Closed system

Species: Daphnia magna

Exposure period: 48 Hours

Results: EC_{50} (24h) = 2.0 mg/l
EC_{50} (48h) = 1.4 mg/l
NOEC = 0.56 mg/l
LOEC = 1.0 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity:>97%

Remarks: The Daphnia magna used in the test were cultured at the ABC facilities. Adult Daphnia were fed an algae and trout chow mixture daily until 24 hours prior to testing. The bioassay was conducted in 250ml glass beakers containing 200 ml of ABC well water. Zero-hour dissolved oxygen concentration was 9.3 mg/l, pH was 8.2, hardness (CaCO3) was 255 ppm, and alkalinity was 368 ppm. Vessels were kept in a water bath at 20°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range-finding experiment was carried out to determine the exposure concentrations for the definitive test. Acetone was used as the solvent for the test solutions, and the experiment included both a control and a solvent control (0.01ml). Concentrations (in duplicate) of the test substance were 0, 0.32, 0.56, 1.0, 1.8 and 3.2 mg/ml. Ten daphnia, first instar less than 24 hours old, were placed in each test chamber. Daphnia in all concentrations were observed once every 24 hours for mortality and abnormal effects. Water quality measurements were monitored throughout the testing and were considered adequate and equivalent to those measurements in the control chamber. Dissolved oxygen concentrations ranged from 9.3-7.4 mg/l (101-80% saturation) and pH ranged from 8.0-8.5. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program

calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.

Reference: Monsanto AB-83X-037, Analytical Bio-Chemistry Labs, 1983

***4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae**

5. TOXICITY

***5.1 ACUTE TOXICITY**

5.1.1 ACUTE ORAL TOXICITY

Type: LD_{50}
Species/strain: Rats, Sprague-Dawley Albino
Value: 271 mg/kg bw for males and females combined
281 mg/kg for males
265 mg/kg for females
# of Animals: 50 (5/sex/dose)
Vehicle: Corn oil
Doses: 200, 313, 490, 767 or 1200 mg/kg bw
Method: Other: Monsanto EHL Protocol, Acute Oral LD50, 1981
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96.09%
Remarks: Groups of five male and five female rats were dosed by oral gavage with the test article as a 392 mg/ml solution in corn oil. Males weighed between 225-247 grams and females weighed between 166-182 grams. Clinical observations were made 3x/day during the first 8 hours, and 2x/day thereafter until sacrifice. Body weights were recorded on days 0, 7 and 14. After a 14-day recovery period, all surviving animals were sacrificed. Necropsies were performed on all animals. Clinical signs of toxicity included lethargy, ataxia, ptosis, and abnormal urine coloration (green and/or reddish-brown). Necropsy findings included gastrointestinal inflammation, which reached the severity of hemmorhage in many cases, gastrointestinal distension, and red, fluid-filled gastric masses. The presence of these masses indicated that the toxicity to gastrointestinal tissue might have contributed to lethality in virtually all rats that died during the test. Previous oral and dermal toxicity studies with this material have noted the corrosivity to tissue that complicates accurate determinations of LD50 values. The acute oral LD50 for each sex and the combined sexes was calculated using the probit analysis method of Finney (1971).

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
200	2/5	1/5	3/10
313	4/5	3/5	7/10
490	1/5	5/5	6/10
767	5/5	5/5	10/10
1200	5/5	5/5	10/10

Reference: Monsanto ML-82-181, Environmental Health Labs, 1983
Reliability: (1) Valid without restriction

Type: LD_{50}
Species/strain: Rats, Sprague-Dawley Albino
Value: 148 mg/kg bw for males and females combined
<200 mg/kg for males
222 mg/kg for females
# of Animals: 50 (5/sex/dose)
Vehicle: None - Undiluted
Doses: 200, 263, 346, 456 or 600 mg/kg bw
Method: Other: Monsanto EHL Protocol, Acute Oral LD50, 1981
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96.09%
Remarks: Groups of five male and five female rats were dosed by oral gavage with the undiluted test article. Males weighed between 211-236 grams and females weighed between 151-174 grams. Clinical observations were made 3x/day during the first 8 hours, and 2x/day thereafter until sacrifice. Body weights were recorded on days 0, 7 and 14. After a 14-day recovery period, all surviving animals were sacrificed. Necropsies were performed on all animals. The acute oral LD50 for female rats was calculated by the method of Thomson and Weil (1952). The acute oral LD50 for male rats and for the combined sexes was calculated by the method of Finney (1971), but the latter two values were lower than any of the doses administered. Lower dosages were not administered in an attempt to attain lethality of less than 50% since the dose volumes would have been very small. It was considered unlikely that such volumes of the neat material could be reliably measured and administered. Commonly observed clinical observations included green and/or red urine, lethargy, ataxia, prostration, salivation and ptosis. At necropsy, signs of gastrointestinal inflammation were observed in 31 of the 40 animals that died following dosing. The stomach appeared hemorrhaged in six of these animals. Fourteen animals had gastrointestinal distension. Eleven rats had green material in the urinary bladder and/or green urinary staining of fur. Seven male and three female rats had diffuse off-white hepatic coloration or multiple white foci on all hepatic lobes. Hemorrhaged diaphrams were observed in four rats. Four animals of each sex had brown and/or clear fluid in the thoracic cavity. Three animals had red fluid in the urinary bladder. Dark adrenals were observed in seven animals. All animals that exhibited any of the above effects died during the test.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
200	4/5	1/5	5/10
263	5/5	5/5	10/10
346	2/5	4/5	6/10
456	5/5	5/5	10/10
600	4/5	5/5	9/10

Reference: Monsanto ML-82-022a, Environmental Health Labs, 1983
Reliability: (1) Valid without restriction

5.1.2 ACUTE INHALATION TOXICITY

Type:	LCL_0
Species/strain:	Rats, Sprague-Dawley Albino
Exposure time:	6 Hours
# of Animals:	No data
Value:	600 mg/m3
Method:	No data
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: "Commercial"
Remarks:	RTECS and NTP reference. Test conditions unknown. No additional data available.
Reference:	Kodak Company Reports, 1971
Reliability:	(4) Not assignable - data from a secondary literature source.

Type:	LC_{50}
Species/strain:	Rats, Sprague-Dawley Albino
Sex:	Male
Exposure time:	6 Hours
Value:	>0.2 mg/l
# of Animals:	6
Method:	A.T.S. 8/1973
GLP:	No
Test substance:	As prescribed by 1.1-1.4, purity: >96%
Remarks:	Six male rats were exposed to the test article at a concentration of 0.2 mg/l at ambient temperature at an airflow rate of 4 l/min for six hours. The test chamber temperature was 27°C, and the chamber humidity was 80%. Test chamber volume was 35 liters. The difference in weight of the sample after the test indicated that 0.4 grams had been vaporized under test conditions. There were no clinical signs of toxicity noted during the experiment. Following a 14-day recovery period, all animals were sacrificed. Necropsy findings were that all viscera examined appeared normal. 95% confidence limits 270-330 mg/kg.
Reference:	Monsanto Y-76-262, Younger Laboratories, 1976
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

5.1.3 ACUTE DERMAL TOXICITY

Type:	LD_{50}
Species/strain:	Rabbits, New Zealand Albino
Value:	2806 mg/kg bw (for both males and females)
# of Animals:	24 (4/sex/dose)
Vehicle:	None
Doses:	2500, 3536, 5000 mg/kg bw
Method:	Other: Monsanto EHL Acute Dermal LD50 Protocol, 1982
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 96.09%
Remarks:	Young adult rabbits weighing between 2.43 and 3.04 were purchased from Isaac's Farm in Litchfield, IL, for this study. Groups of four male and female rabbits per dose level were exposed to the test compound via a single dermal application to shaved skin. Two animals from each group were predesignated to have their skin abraided in the treatment area. Skin of the other animals was intact. Clinical observations were made 3x/day during the first eight hours after exposure, then 2x/day thereafter

until sacrifice. Necropsies were performed on all animals. Clinical signs of toxicity included lethargy, ataxia, green coloration of the urine, partial loss of ability to move the limbs, and localized dermal effects attributed to the direct contact between skin and test article. Death occurred in the same number of male and female animals, and in the same number of rabbits with intact and abraded skin. In addition to these effects, body weight loss occurred in three of the six survivors during the first week of testing. All six of these animals gained weight during the second week. Findings on necropsy included green material in the bladder of sixteen animals, four animals with an enlarged gall bladder, and five with hepatic discoloration. Determination of the acute dermal LD50 for each sex and for the combined sexes was made using the method of Thomson and Weil (1952).

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
2500	1/4	1/4	2/8
3536	4/4	4/4	8/8
5000	4/4	4/4	8/8

Reference: Monsanto ML-82-022b, Environmental Health Lab, 1983
Reliability: (1) Valid without restriction

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

Species/strain: Rabbits, New Zealand White
Results: Corrosive
Classification: Corrosive (causes burns)
# of Animals: 6
Vehicle: None
Doses: 0.5 ml
Method: Draize, J.H. Woodard, G., and Calvery, H.O., Methods for the Study of Irritation and Toxicity of Substances Applied Topically To the Skin and Mucous Membranes, J. Pharmacol. Exp. Therap. 82: 377-390, 1944
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96.09%
Remarks: The undiluted test article, at a volume of 0.5 ml, was applied to the intact and abraded shaved skin of six rabbits for 24 hours. The initial observation was made approximately one hour after exposure. Dermal irritation was scored by the Draize Method, and results recorded on day 1, 3, 7, 10, 14 and 17 after exposure. Scarring, hardening of the skin, scabbing and sloughing skin were noted on all animals. The test article was classified as corrosive under the test conditions.
Reference: Monsanto ML-82-022c, Environmental Health Lab, 1983
Reliability: (1) Valid without restriction

Species/strain: Rabbits, New Zealand Albino
Results: Highly irritating
Classification: Irritating

# of Animals:	6
Vehicle:	None
Doses:	0.5 ml
Method:	D.O.T. Hazardous Material Regulations 49 CFR 173.240, 1976
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: not stated
Remarks:	The undiluted test article was applied to the shaved skin of six rabbits in a single application of 0.5 ml. The test site was covered for four hours with surgical gauze and an elastic bandage. The entire trunk of the rabbit was wrapped in 2 mil thick plastic to prevent evaporation of the test article, and the plastic was covered with a white cotton towel. After four hours, the wrappings were removed, and the skin allowed to equilibrate for hydration and compression for 30 minutes. Skin was scored for erythema, eschar formation and corrosion in accordance with the Federal Hazardous Substances Act Grading Code, 16 CFR 1500.41. After grading, the test site was washed with water. Test sites were scored again after 24, 48 and 72 hours, and 1 and 2 weeks. Gross observations of corrosion were noted in 2/6 rabbits at I week and in 4/6 rabbits after 2 weeks. Under the conditions of the DOT test, these results were judged to be between "marginal" and "severely irritating but not corrosive". Because of the results of earlier studies, the manufacturers of this material have chosen to classify it as "corrosive" for both use and transportation.
Reference:	Monsanto XX-84X-144, Gulf South Research, 1983
Reliability:	(1) Valid without restriction

5.2.2 EYE IRRITATION/CORROSION

Species/strain:	Rabbits, New Zealand Albino
Results:	Corrosive
Classification:	Risk of serious damage to eyes
# of Animals:	6
Vehicle:	None
Doses:	0.1 ml
Method:	Draize et.al., J. Pharmacol., Exp. Therap. 82: pp 377-390, 1944
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity 96.09%
Remarks:	A single dose of 0.1 ml of the undiluted test article was placed in the one eye of three male and three female rabbits, with the untreated eye serving as the control. A topical anesthetic was available if discomfort appeared severe. Signs of irritation were scored according to the Draize procedure. Scoring will be done at 24, 48 and 72 hours after treatment. Discomfort on application was slight. Observations at 24 hours included severe erythema with necrosis, severe edema, copious discharge containing a whitish exudate and severe swelling of conjunctivae. Under the test conditions, the material was classified as "corrosive". Scabs sloughed off in 14 to 21 days with no apparent permanent corneal damage.
Reference:	Monsanto ML-82-022d, Environmental Health Laboratory, 1983
Reliability:	(1) Valid without restriction

*5.4 REPEATED DOSE TOXICITY

Species/strain: Rats, Sprague-Dawley Albino
Sex: Male/Female
Route of Administration: Oral gavage
Exposure period: 28 days
Frequency of treatment: Daily
# of Animals: 100 (10/sex/dose)
Post exposure observation period:
Dose: 0, 10, 25, 50, or 100 mg/kg
Control group: Yes, Concurrent vehicle
NOEL: Not determined
LOEL: 10 mg/kg
Results: 100 male and female rats (10/sex/dose level) were dosed with the test article in corn oil vehicle at the above levels for a period of 28 days. The animals were observed 2x/day for mortality or signs of toxicity. Detailed observations, body weights and feed consumption were documented 1x/week. Hematology determinations and clinical chemistry determinations were made on all control animals and the high-dose animals prior to terminal sacrifice. Major organs were weighed at necropsy to calculate mean absolute weights and organ-to-body weight ratios. Select tissues/organs from all animals were retained in 10% neutral buffered formalin at necropsy. Liver sections from all animals were subsequentially examined histologically. Additional clinical chemistry determinations of GGTP, SGOT, SGPT, Bilirubin, SAP and 5-nucleotidase were performed on all treated animals. A complete gross necropsy was performed on all animals at sacrifice and within 16 hours of any animal who died during the course of the study. Two mid-dose males died within the first week of treatment and two high-dose females died during week 3. Cause of death did not appear to be treatment-related. One additional mid-dose female was sacrificed at day 15 following an injury during dosing. All other animals survived to sacrifice. Gross necropsy findings on two high-dose females was a slightly pale liver. In males, a finding of dilation of the right renal pelvis was found in several animals at all dose levels, including controls. Adverse effects observed included increased liver weights and elevation of serum enzymes SGOT, SGPT and GGTP, indicative of hepatocellular damage, as well as a dose-dependent increase in the incidence of hepatocellular lesions. Because the results of this study demonstrated hepatic effects in both sexes and at all treatment levels, a No Observed Effect Level could not be established. Data collected during the study were statistically evaluated using Student's t-test at the 95% confidence level to determine which means were significantly different from the corn-oil treated controls. Data analyzed statistically during the study included body weight, feed consumption, clinical chemistry, hematology, organ weights, and organ-to-body weight ratios.
Method: OECD Guidelines for the Testing of Chemicals, 1981
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97.0%
Reference: Monsanto PR-83-317, Pharmacopathics Research Labs, 1984
Reliability: (1) Valid without restriction

Species/strain:	Rats, Sprague-Dawley Albino
Sex:	Male/Female
Route of Administration:	Oral dietary
Exposure period:	90-94 days
Frequency of treatment:	Daily
Post exposure observation period:	
Dose:	0, 20, 100 or 500 ppm
Control group:	Yes, Concurrent no treatment
NOEL:	100 mg/kg
LOEL:	500 mg/kg
Results:	In a subchronic feeding study, groups of male and female rats were fed the test article via dietary admixture for three months. After 65 days of treatment, the low-dose (20 ppm) group was increased to 1000 ppm for twenty-five days, and then to 2000 ppm for the final four days of the study. Findings included decreased body weights and body weight gain in the 500 ppm males, and decreased body weights in the 500 ppm females. There were no clinical signs of toxicity noted for any dose level for either sex. All animals survived until terminal sacrifice. Hematology determinations and clinical chemistry determinations were made on all animals prior to sacrifice, and all animals received a complete gross necropsy. There were no hematological or histopathological findings at any dose level that were considered to be treatment-related. The NOEL was determined to be 100 ppm, or 6.6 mg/kg/day, for both males and females based upon the reduced body weights seen at 500 ppm.
Method:	No data
GLP:	No data
Test substance:	As prescribed by 1.1-1.4 purity: Commercial grade >96%
Reference:	E.I. DuPont de Nemours, unpublished data, 1987
Reliability:	(4) Not assignable. Data from a secondary literature source

*5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

Type:	Bacterial Reverse Mutation - Ames
System of testing:	Salmonella typhimurium TA97, TA98, TA100, TA1535, TA 1537, TA1538
Concentration:	No data
Metabolic activation:	With and without
Results:	
Cytotoxicity conc:	With metabolic activation: Not determined Without metabolic activation: Not determined
Precipitation conc:	Not determined
Genotoxic effects:	
	With metabolic activation: Negative Without metabolic activation: Negative
Method:	OECD 471 Plate Overlay method
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: Technical grade
Remarks:	The test compound was tested in Ames/Salmonella plate

incorporation assays using the tester strains TA 97, TA98, T A100, TA1535, and TA1538 and TA1537 in the presence and absence of an Aroclor-induced rat liver mammalian metabolic activation system (S-9 Mix). No mutagenic activity was observed for the test compound in any of these assays.

Reference: Zeiger, et. al., Environ. Mol. Mutagen, 1998

Reliability: (4) Not assignable - data from a secondary literature source

B. NON-BACTERIAL IN VITRO TEST

Type: Cytogenetics Assay .

System of testing: Cultured Chinese hamster ovary (CHO) cells and cultured Chinese Hamster Lung (CHL) cells

Concentration: No data

Metabolic activation: With and without

Results:

Cytotoxicity conc: With metabolic activation: Not determined
Without metabolic activation: Not determined

Precipitation conc: Not determined

Genotoxic effects:

With metabolic activation (CHO):	Negative
Without metabolic activation (CHO):	Negative
With metabolic activation (CHL):	Negative
Without metabolic activation (CHL):	Equivocal

Method: OECD 473 – *in vitro* Mammalian Chromosomal Aberration Test

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity: Commercial grade

Remarks: The test article was one of 25 chemicals tested for the induction of chromosomal aberrations in two cultured mammalian cell systems the cultured cells from Chinese hamster ovaries (CHO, and those from Chinese hamster lungs (CHL), in the presence absence of metabolic activation with the S9 mix. The test article negative with metabolic activation in both CHO and CHL cells, and negative without metabolic activation in CHO cells. The results for CHL cells without metabolic activation were equivocal. Overall, the results indicate that the test article is negative for the potential to cause chromosomal aberrations, both with and without metabolic activation, under the test conditions.

Reference: Sofuni, et.al. Mutation Research, 1990

Reliability: (4) Not assignable - data from a secondary literature source

*** 5.6 GENETIC TOXICITY IN VIVO**

***5.8 TOXICITY TO REPRODUCTION**

***5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY**

5.10 OTHER RELEVANT INFORMATION

*** 5.11 EXPERIENCE WITH HUMAN EXPOSURE**

Results: Cyanosis and anemia have been observed in workers involved in the manufacture of Antioxidant 22.

Remarks: Dermal route

Reference: E,I, DuPont de Nemours, 1987

Results: Historically, three incidents involving accidental human overexposure involving Antioxidant 22 have been documented. Skin reactions noted were irritation and a pigmented crust that scaled away in a few days, leaving an erythematous base. Systemic reactions, indicative of skin absorbtion, included profuse perspiration, slow pulse, and a general feeling of anxiety.

Remarks: Data from 1945 does not reflect current industrial practice utilizing Impervious gloves and other personal protective equipment

Reference: Kendrick, M.C., The Medical Bulletin, 1945

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3081-14-9
1,4-Benzenediamine, N,N'-bis(1,4-dimethylpentyl)-

Molecular Weight: **304.52**
Molecular Formula: **C20-H36-N2**

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance: Organic
B. Physical State: Dark reddish brown oily liquid
C. Purity: 95-98 % Typical for Commercial Products

1.2 SYNONYMS

Santoflex® 77PD
Santoflex® 77
Flexzone® 4L
Naugard® 12
UOP 788®
Vulkanox® 4030
N,N'-Bis(1,4-dimethylpentyl)-p-phenylenediamine

1.3 IMPURITIES

77PPD isomers <1%
Dialkylated phenylenediamines <2%
Monoalkylated phenylenediamines <2%

1.4 ADDITIVES None

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	-36 °C
Decomposition:	No
Sublimation:	No
Method:	Not Specified
GLP:	No data
Remarks:	None
Reference:	NTP Chemical Repository 1990
Reliability:	(4) Not assignable – data from secondary literature source

*2.2 BOILING POINT

Value:	>350°C
Pressure:	1013 hPa
Decomposition:	No
Method:	Instrumental – DSC Thermal Stability, 2002
	.
GLP:	Yes
Remarks:	Sample was run from ambient temperature to 350° at 10°/minute Straight baseline with no endotherm after melt, indicating thermal stability.

Reference:	Flexsys Analytical Research Report AP2002.118, 2002
Reliability:	(1) Valid without restriction
Value:	364.35°C
Pressure:	1013 hPa
Decomposition:	No data
Method:	MPBPWIN v1.40
	.
GLP:	No
Remarks:	Estimation based on molecular structure and measured values for melting point, vapour pressure and Log Kow. Good agreement with measured DSC value above.
Reference:	EPISUITE/EPIWIN MPBPWIN v1.40
Reliability:	(2) Valid with restrictions – modelling data
Value:	183-185 °C
Pressure:	1.3332 hPa
Decomposition:	No
Method:	No data
GLP:	No data
Remarks:	Boiling point at reduced pressure (1mm Hg)
Reference:	Monsanto Physical Constants of CP25447 (SMP 1977)
Reliability:	(2) Valid with restrictions – no method detail

†2.3 DENSITY (relative density)

Type:	Density
Value:	0.89-0.91
Temperature:	25 °C
Method:	Flexsys Standard Method of Analysis FF97.4-1
GLP:	Yes
Remarks:	Hydrometer method. Hydrometer must meet standards set in ASTM-E-100
Reference:	ASTM D891-94 method equivalent
Reliability:	(1) Valid without restrictions

***2.4 VAPOUR PRESSURE**

Value:	0.0000015 hPa
Temperature:	25°C
Method:	measured Gas Saturation Method, W.F. Spencer and M.M. Cliath, Environ. Sci. Tech. 3, 670 (1969)
GLP:	Yes
Remarks:	Nitrogen carrier gas, Tenax-GC sorbent, GC analysis
Reference:	Monsanto SRI 8669, SRI International, 1980
Reliability:	(1) Valid without restriction
Value:	0.0799 hPa @ 147°C 0.2533 hPa @ 160°C

	1.1732 hPa @ 180°C
	4.2663 hPa @ 200°C
Method:	No data
GLP:	No data
Remarks:	Pressures determined for expected process temperatures
Reference:	Monsanto Report # MAK004, January, 1983
Reliability:	(2) Valid with restrictions – lack of method detail

***2.5 PARTITION COEFFICIENT $log_{10}P_{ow}$**

Log Pow:	5.34 log P
Temperature:	22°C
Method:	measured
	EPA Federal Register Vol. 44, No. 53 (1979)
GLP:	Yes
Remarks:	Octanol used as solvent
Reference:	Monsanto SRI 8669, SRI International, 1980
Reliability:	(1) Valid without restriction

***2.6 WATER SOLUBILITY**

A. Solubility

Value:	21 ug/ml @ pH 5
	0.8 ug/ml @ pH 9
Temperature:	22°C
Description:	Of very low solubility
Method:	May, W.E., Wasik, S.P., Freeman, D.H., Anal. Chem. 50 (1) 175-178, 1978
GLP:	Yes
Remarks:	May Method chosen for low-solubility chemicals; solubility at pH 7 was not measured due to time and equipment constraints. Solubility at pH 5 was (+/-) 6.8. Solubility at pH 9 was (+/-) 0.1
Reference:	Monsanto SRI 8669, SRI International, 1980
Reliability:	(1) Valid without restriction

Value:	1.242 mg/l
Temperature:	25°C
Description:	Of very low solubility
Method:	WSKOW v1.40
GLP:	No
Remarks:	Calculation based on molecular structure and measured values for Melting point, vapour pressure and Log Kow. Good agreement with measured values at different pHs above.
Reference:	EPISUITE/EPIWIN WSKOW v1.40
Reliability:	(2) Valid with restrictions – modelling data

B. pH Value, pKa Value

pH Value:	Not Applicable

2.7 FLASH POINT (liquids)

Value:	182 °C
Type of test:	Open cup
Method:	ASTM D 92 Cleveland Open Cup
GLP:	Yes
Remarks:	No method deviations
Reference:	American Society for Testing and Materials (ASTM), 1997
Reliability:	(1) Valid without restriction

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

B. Other data – Henry's Law Constant

Results:	3.549E-007 atm-m3/mole
Remarks:	Calculated at 25°C using measured values for melting point, vapour pressure and Log Kow.
Reference:	EPIWIN/HENRYWIN v3.10
Reliability:	(2) Valid with restrictions – modelling data

3. <u>ENVIRONMENTAL FATE AND PATHWAYS</u>

3.1 STABILITY

Type:	Abiotic (hydrolysis)
Half life:	Not Determined
Degradation:	97% at pH 7.0 at 25 °C after 24 Hours
Method:	Extraction, ABC Protocol M-8305 (1985)
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >95%
Remarks:	Primary stock solutions of 1.00 mg/l of the test compound were prepared in nanograde acetone. Subsequent dilutions for spiking and gas chromatography standards were also prepared in nanograde acetone. Test samples were extracted with three 75ml portions of methylene chloride. The extracts were dried by passing them through a funnel containing anhydrous sodium sulfate. No test substance detected at seven days. Hydrolysis products identified by GC analysis and confirmed by GS/Mass Spectrometry as 4-hydroxydiphenylamine (30%) and Benzoquinoneimine-n-phenyl (70%). The Benzoquinoneimine-n-phenyl is the oxidized form of 4-hydroxydiphenylamine (CAS# 122-37-2, C12-H11-N-O). The amine portion of the test compound molecule was not isolated, nor was it apparent from the GC-MS spectra. It was postulated that the amine portion might be present in the hydrolysis water layer, indicating that the linkage was cleaved at the aromatic carbon-nitrogen bond.
Reference:	Monsanto ABC 32303, Analytical Bio-Chemistry Labs, 1986
Reliability:	(1) Valid without restriction

***3.1.1 PHOTODEGRADATION**

Type:	Water
Light source:	Sunlight
Light spectrum:	Natural sunlight, March 7, 1980
Relative intensity:	No data

Spectrum of substance: 262 nm
Concentration of Substance: 5ppm
Temperature: 0°C and 23 °C
Direct photolysis:
Half life: 2 hours (light) and 4 hours (dark)
Degradation: No data
Quantum yield: No data
Method: measured
Direct Photolysis
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: >94%
Remarks: Solutions of 5ppm of the test compound were prepared in purified water using 3.5% methanol as a cosolvent. Solutions were placed in borosilicate tubes and exposed to sunlight at midday. Dark controls were maintained at 23°C. Photolyzed solutions were maintained at 0°C and all samples were analyzed on the same day. 1ml of 0.1N NaOH was added to 5ml of the photolized solution and then extracted with methylene chloride. Methylene chloride extracts were combined and brought up to a volume of 4ml for direct injection into a GC for analysis.
Reference: Monsanto SRI 8669, SRI International, 1980
Reliability: (1) Valid without restriction

***3.1.2 STABILITY IN WATER**

***3.3.1 TRANSPORT**

Type: Volatility
Media: Water
Method: Estimation Method, 1990
Results: Volatilization half-life from model river: 1.051E+004 hours
Volatilization half-life from model lake: 1.148E+005 hours
Volatilization Constant from water: 1.78E-008 atm-m3/mole
Remarks: Model river = 1 m deep flowing at 1 m/sec and wind velocity of 3 m/sec.
Model lake = 1 m deep flowing at 0.05 m/sec and wind velocity of 0.5 m/sec.
Reference: Handbook of Chemical Property Estimation Methods, 1990
Reliability: (2) Valid with restrictions – modelling data

***3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)**

Media: Air-biota-sediment-soil-water
Method: Fugacity level III
EPIWIN v3.10
Results:

	Mass Amount (%)	Half-life (hrs)	Emissions (kg/hr)
Air	0.0904	2.04	1000
Water	14.9	900	1000
Soil	47.3	900	1000
Sediment	37.7	3.6E+003	0

Persistence time estimated at 977 hours
Remarks: Calculations based on molecular structure and measured values for melting point, vapour pressure and Log Kow.
Reference: EPISUITE/EPIWIN v3.10

Reliability: (2) Valid with restrictions – modelling data

***3.5 BIODEGRADATION**

Type: aerobic
Inoculum: adapted
Concentration of the chemical: 24-25 mg/l related to test substance
Medium: Sewage/soil/sludge mixture
Degradation: Yes
Results: inherently biodegradable
Kinetic 50 % in 35 days
Method: ASTM Proposed Standard for the Determination of the Ultimate Biodegradability of Organic Chemicals, 1979
GLP: No
Test substance: As prescribed by 1.1-1.4, purity: >94%
Remarks: The ultimate degradation of the test compound was assessed using a carbon dioxide evolution shake flask procedure. The procedure was run in triplicate, with 24-25 mg/l of the test compound added to 100 ml of acclimated bacterial innoculum and 900 ml minimal salts media. A sterile control was also employed. For sterile controls, 100 mg/l HgCl2 is also added. Theory carbon values were determined experimentally using a Perkin-Elmer 240 Elemental Analyzer. CO2 evolution was determined via titration. There was no significant biodegradation noted under sterile conditions. Results of the triplicate runs gave 37%, 58% and 56% of theory CO2 evolution, for a mean value of 50%. This indicates that long-term environmental persistence of the parent compound or any metabolites is not likely.
Reference: Monsanto ES-79-SS-25 MIC Environmental Sciences, 1979
Reliability: (1) Valid without restriction

4. ECOTOXICITY

***4.1 ACUTE/PROLONGED TOXICITY TO FISH**

Type of test: static
Closed system
Species: Salmo gairdneri (Rainbow Trout)
Exposure period: 96 hours
Results: LC_{50} (24h) = 51 ug/l
LC_{50} (48h) = 39 ug/l
LC_{50} (96h) = 32 ug/l
NOEC = 20 ug/l
LOEC = 32 ug/l
Analytical monitoring: No
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

.
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >99%
Remarks: The test material, in reagent-grade Acetone, was introduced into 15 liters

	of diluent water in all-glass vessels. Nominal test concentrations (duplicate) were 0, 24, 32, 42, 56, 75 or 140 ug/l, plus a solvent (acetone) control. To each test vessel, 10 rainbow trout, standard length 3.7 cm, were then added. The test fish were not fed 48 hours prior to testing, nor during exposure. No aeration was provided during the test, and temperature was maintained at 12°C. Dissolved oxygen ranged from 9.7 mg/l (91% saturation) to 2.4 mg/l (22% saturation) from beginning to end of exposure, respectively. pH values ranged from 7.2 initially, to 6.8 at the end of the test. Observations and mortality counts were made every 24 hours. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50s and the 95% confidence intervals were calculated from the regression equation.
Reference:	Monsanto BN-76-254, EG&G Bionomics, 1976
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

Type of test:	static Closed system
Species:	Lepomis machrochirus (Bluegill Sunfish)
Exposure period:	96 hours
Results:	LC_{50} (24h) = 261 ug/l LC_{50} (48h) = 201 ug/l LC_{50} (96h) = 182 ug/l NOEC = 140 ug/l LOEC = 180 ug/l
Analytical monitoring:	No
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

	.
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: >99%
Remarks:	The test material, in reagent-grade Acetone, was introduced into 15 liters of diluent water in all-glass vessels. Nominal test concentrations (duplicate) were 0, 140, 180, 240, 320 or 560 ug/l, plus a solvent (acetone) control. To each test vessel, 10 bluegill, standard length 3.8 cm, were then added. The test fish were not fed 48 hours prior to testing, nor during exposure. No aeration was provided during the test, and temperature was maintained at 22°C. Dissolved oxygen ranged from 8.8 mg/l (100% saturation) to 0.2 mg/l (2% saturation) from beginning to end of exposure, respectively. pH values ranged from 7.3 initially, to 6.8 at the end of the test. Observations and mortality counts were made every 24 hours. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50s and the 95% confidence intervals were calculated from the regression equation.
Reference:	Monsanto BN-76-254, EG&G Bionomics, 1976
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

Type of test:	static

	Closed system
Species:	Pimephales promelas (Fathead Minnows)
Exposure period:	96 hours
Results:	LC_{50} (24h) = 0.32 mg/l LC_{50} (48h) = 0.28 mg/l LC_{50} (96h) = 0.28 mg/l NOEC = Not Determined LOEC = 0.10 mg/l
Analytical monitoring:	No
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
	.
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >99%
Remarks:	Test fish were obtained from an ABC Laboratories in-house culture. Test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations was maintained during the holding period, during which time the fish were fed a standard diet of commercial fish food until 48 hours prior to testing, when feeding was stopped. Test fish had a mean weight of 0.23 g and a mean standard length of 25 mm. The test was conducted in 5-gallon glass vessels containing 15 liters of laboratory well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 mg/l, hardness (CaCO3) of 255 ppm, alkalinity of 368 ppm, and pH 8.2. The test vessels were kept in a water bath at 22°C. Test fish were acclimated to the dilution water and test temperature, and held without food for 48 hours prior to testing. Nanograde Acetone was used to prepare the test solutions and as the solvent control (1.0 ml). Test concentrations were 0, 0.10, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2 or 5.6 mg/l for the test compound. Fish were placed in the testing vessels within 20 minutes of the addition of the test material aliquots. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen values and pH ranges were monitored during the testing and remained within acceptable limits of 100-40% saturation (9.3-3.8 mg/l) for dissolved oxygen and pH value (8.3-8.0) consistent with control. The ammonia concentration was below the toxic limit. Water hardness (CaCO3) was 255 ppm. As a quality check, test fish were challenged with Antimycin A. The estimated 96Hr LC50 and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.
Reference:	Monsanto AB-79-1384361-1a, Analytical BioChemistry Labs, 1979
Reliability:	(1) Valid without restriction
Type of test:	flow-through, dynamic acute
	Open system
Species:	Pimephales promelas (Fathead Minnow)

Endpoint:	LC50 / growth and survival
Exposure period:	14 days
Results:	LC_{50} (14d) = 0.067 mg/l NOEC = 0.018 mg/l LOEC = 0.046 mg/l
Analytical monitoring:	Yes
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975) and Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 99+%
Remarks:	A dynamic 14-day toxicity study was conducted to determine the lethal threshold of the test compound to fathead minnows. Test fish were obtained from Fattig Fish Hatchery in Brady, Nebraska. Fish were held in culture tanks for fourteen days prior to testing on a 16-hour daylight photoperiod. During the holding, acclimation and test periods, test fish received a standard commercial fish food in an amount equivalent to 3% of body weight. Fathead minnows used had an initial mean weight of 0.61g and an initial mean standard length of 33mm. As a quality check, the fathead minnows were challenged with the reference compound Antimycin A. The observed 96hr LC50 and 95% confidence limits were within the expected ranges, indicating that the test fish were in good condition. Twenty fish/dose level were used for the experiment. A flow-through proportional diluter system was used to maintain constant test concentrations by providing intermittent introduction of the test compound and diluent water into the test aquaria. Aerated well water (DO = 9.2ppm, pH = 7.8, hardness = 255ppm, alkalinity = 368ppm) was delivered to the glass aquaria at a rate of 200ml/min/aquarium, an amount sufficient to replace the 30 liter test volume at least 10x/24hr. The test aquaria were maintained at 22°C. Stock solutions were prepared in methanol using 10g/l ascorbic acid as a preservative. Stock solutions were changed daily. The control aquarium received a methanol/ascorbic acid aliquot equivalent to the highest amount of these materials used in the test aquaria. Nominal concentrations of the test compound were 0.04, 0.08, 0.15, 0.28 and 0.50 mg/l. Exposure concentrations were measured by gas chromatography to determine that actual test concentrations on Day 0, 1, 5, 10 and 14. The mean measured levels were 0.018, 0.046, 0.11, 0.22 and 0.45 mg/l, or 50-90% of the nominal values. ONLY THE MEASURED VALUES WERE USED IN THE STATISTICAL CALCULATIONS OF LC50 VALUES. A computerized LC50 program developed by Stephan et al was used to determine the LC50 values and the 95% confidence limits. Behavior observations throughout the test indicated that mortality was preceded by surfacing and loss of equilibrium. Weight measurements of surviving fish at the end of the study yielded the following weight percentages of the control group mean weight: 0.018 mg/l = 84%, and 0.046 mg/l = 81%. An apparent lethal threshold of the test substance to fathead minnows was determined to be 0.067 mg/l and was reached after 12 days as indicated by a cessation in mortality from days 12-14. Water quality parameters of temperature (21-22°C), DO (8.8-7.2 mg/l), pH (7.8-8.0) and ammonia (0.20-0.52 mg/l) were monitored throughout the test and remained within acceptable limits.
Reference:	Monsanto AB-80-1803058-B1, Analytical BioChemistry Labs, 1981
Reliability:	(1) Valid without restriction

Type of test:	flow-through time-independent bioassay Open system
Species:	Pimephales promelas (Fathead Minnow)
Endpoint:	LC50 / Growth and survival
Exposure period:	14 days (336 hours)
Results:	LC_{50} (24h) = 0.07 mg/l LC_{50} (96h) = 0.06 mg/l LC_{50} (14d) = 0.05 mg/l NOEC = Not Determined LOEC = Not Determined
Analytical monitoring:	Yes
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975) and Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 99+%
Remarks:	The test was performed in duplicate 19 liter glass aquaria under flow-through conditions using a Mount-Brungs diluter. Test fish were juvenile fathead minnows reared at SRI and distributed randomly among the test containers at 20 fish/replicate aquaria. The diluter flow was set to provide five tank volumes/day. Stock solutions prepared in acetone and stabilized with ascorbic acid. During the test, fish were fed frozen brine shrimp at a rate equal to 5% of body weight. Stock solutions were prepared by adding 0.72 ml of the test compound to 125 ml of acetone. This solution was metered into the diluter at 3.0 ml/hour. A separate bottle was used to supply a dilute acetone solution to the solvent controls to obtain a nominal concentration of 150 ul/liter. Nominal concentrations of the test solution were 0.00, 0.03, 0.06, 0.12, 0.25 and 0.50 mg/liter, in addition to the solvent control. The test was terminated after 14 days of exposure, as no deaths had occurred during the preceding 48 hours. Dissolved oxygen, pH, temperature and chemical concentrations were monitored routinely, alternating between the replicates. Actual chemical concentrations were measured by an internal standard GC method. The actual chemical concentrations were less than the nominal concentrations, although high enough to produce mortality. The variability was attributed to instability of the test compound in water and possibly to incomplete dispersion. Measured concentrations were 0.00, 0.03, 0.03, 0.05, 0.10 and 0.17. ONLY THE MEASURED VALUES WERE USED IN STATISTICAL CALCULATIONS FOR LC50 VALUES. Ranges for water quality parameters during the study were 5.9-8.5 mg/liter for DO, 7.0-7.8 for pH, and 21.2-21.8°C for temperature. Average fish length was 2.68cm and weight was 0.15g. The probit method was used for calculating the LC50 values and the 95% confidence limits.
Reference:	Monsanto SR-80-1803058-A1, SRI International, 1981
Reliability:	(1) Valid without restriction

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test:	static Closed system
Species:	Daphnia magna

Exposure period:	48 hours
Results:	$EC_{50\ (24h)}$ = 0.44 mg/l $EC_{50\ (48h)}$ = 0.37 mg/l NOEC = 10 mg/l
Analytical monitoring:	No
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >96%
Remarks:	The Daphnia magna used in the test were cultured at the ABC facilities. Adult Daphnia were fed an algae and trout chow mixture daily until 24 hours prior to testing. The bioassay was conducted in 500 ml glass beakers containing 250 ml of ABC well water. During the test, dissolved oxygen concentration ranged from 9.2-7.4 mg/l, pH range was 8.1-9.1, hardness (CaCO3) was 255 mg/l, and alkalinity was 368 mg/l. Vessels were kept in a water bath at 20°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range-finding experiment was carried out to determine the exposure concentrations for the definitive test. Acetone was used as the solvent for the test solutions, and the experiment included both a control and a solvent control (0.01ml). Concentrations (in duplicate) of the test substance were 0, 0.1, 0.18, 0.31, 0.56 and 1.0 mg/l. Ten daphnia, first instar less than 24 hours old, were placed in each test chamber. Daphnia in all concentrations were observed once every 24 hours for mortality and abnormal effects. Water quality measurements were monitored throughout the testing and were considered adequate and equivalent to those measurements in the control chamber. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.
Reference:	Monsanto AB-79-1384361-1b, Analytical Bio-Chemistry Labs, 1979
Reliability:	(1) Valid without restriction

C. Other aquatic organisms

Type of test:	static Closed system
Species:	Paratanytarsus parthenogenetica (Midge)
Exposure period:	48 hours
Results:	$EC_{50\ (24h)}$ = 4.4 mg/l $EC_{50\ (48h)}$ = 1.7 mg/l NOEC = 0.56 mg/l
Analytical monitoring:	No
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >94%
Remarks:	Test midge for this study were cultured at the ABC facilities. The adult midge were fed a suspension of trout chow and alfalfa daily until 24 hours prior to testing. The test was carried out using 3rd and 4th instar larvae, 8-10 days old. The static bioassay was conducted in 250 ml glass beakers containing 200 ml of ABC well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.2 mg/l, hardness

(CaCO3) of 255 ppm, alkalinity (CaCO3) of 368 ppm and pH 7.8. The test vessels were kept in a water bath at 20°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range finding experiment preceded the definitive bioassay. Nanograde Acetone was used to prepare the test solutions of 0, 0.56, 1.0, 1.8, 3.2, 5.6, 10.0 or 18.0 mg/l, and as the solvent control. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen content ranged from 8.9 to 7.8 mg/l and pH ranged from 7.9 to 8.4 during the testing. Water quality parameters of temperature, dissolved oxygen content and pH were measured at the termination of the test and were within acceptable limits. The LC50 values were calculated via a computerized program performing the following statistical tests: binomial, moving average and probit tests.

Reference: Monsanto AB-81-9AB981014, Analytical BioChemistry Labs, 1981

Reliability: (1) Valid without restriction

***4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae**

Species: Selenastrum capricornutum (Freshwater alga)

Endpoint: Biomass and Growth rate

Exposure period: 96 hours

Results: EC_{50} (24h) = >200 mg/l
EC_{50} (48h) = >120<200 mg/l
EC_{50} (72h) = 86 mg/l
EC_{50} (96h) = 52 mg/l
NOEC = Not Determined
LOEC = Not Determined

Analytical monitoring: No

Method: EPA Selenastrum capricornutum Algal Assay Test 1978
Closed system

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 99+%

Remarks: The test algae were obtained from the US EPA Environmental Research Laboratory in Corvallis, Oregon. Beginning cell numbers in the test flasks were 1.0 x 10(4) cells/ml. Cultures were incubated at 24°C under approximately 4,300 lux illumination. Triplicate cultures were employed for each of the test concentrations and the control. Test containers were 125ml flasks containing 50ml of test medium. Concentrations for the definitive test were based on the results of a 72-hr range-finding study. These concentrations were 0, 26, 43, 72, 120 and 200 ppm. Reagent-grade Dimethylformamide (DMF) was used to prepare the stock solutions and as the solvent control, maximum volume 0.05 ml DMF. The pH values ranged from 7.5 at the beginning of the study, to 7.3 at the 96-hour mark. There were no other water quality measurements reported in this study. Statistical analysis involved converting each test concentration to a logarithm, and the corresponding percentage decrease of in vivo chlorophyll a or cell numbers was converted to a probit (Finny, 1971). The EC50s and 95% confidence limits were then calculated by linear regression.

Reference: Monsanto BN-79-1384361-2, EG&G Bionomics, 1979

Reliability: (2) Valid with restrictions – lack of water quality data

4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

4.5.1 CHRONIC TOXICITY TO FISH

5. TOXICITY

***5.1 ACUTE TOXICITY**

5.1.1 ACUTE ORAL TOXICITY

Type:	LD_{50}
Species/strain:	Rats, Sprague-Dawley Albino
Value:	730 mg/kg bw
Sex:	Male and female
# of Animals:	20
Vehicle:	None - undiluted
Doses:	501, 631, 794 or 1000 mg/kg bw
Method:	Single Oral Dose, Younger Laboratories Protocol, 1973
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: 96%
Remarks:	Four groups of male and female rats (5 animals/dose level) were fed a single oral dose of the undiluted test article via oral gavage. Male rats had initial average body weights of 205-235 grams: females had initial average body weights of 215-235 grams. Dosages were 501, 631, 794 and 1000 mg/kg. Clinical signs of toxicity included reduced activity and appetite for four to six days for survivors, and increasing weakness, collapse and death for decedents in two to seven days, with most deaths occurring in four days. Gross autopsy findings on decedents were hemorrhagic areas in the lungs, discolored livers and acute gastrointestinal inflammation. Survivors were sacrificed after ten days. All viscera of survivors appeared normal. 95% confidence limits 690-770 mg/kg.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
501	1/2	0/3	1/5
631	1/3	0/2	1/5
794	1/2	2/3	3/5
1000	3/3	2/2	5/5

Reference:	Monsanto Y-73-168 Younger Laboratories, 1973
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

5.1.2 ACUTE INHALATION TOXICITY

Type:	LC_{50}
Species/strain:	Rats, Sprague-Dawley Albino
Exposure time:	6 hours
Sex:	Male
# of Animals:	6
Value:	Not determined; sample did not vaporize
Method:	Acute Inhalation LC50, Younger Laboratories Protocol, A.T.S. 1973
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: 96%
Remarks:	Male rats were exposed to the test article in an inhalation chamber for a period of six hours at ambient temperature of 26°C. Chamber capacity was 35 liters, relative humidity was 85%, and the airflow rate was 4.0 l/minute.

The initial sample size of the test article was 133 grams. At the end of six hours, the sample was reweighed and found to be 133 grams, and no sample was recovered from the chamber air condenser. The test compound did not vaporize under the test conditions. No animal experienced any symptoms of toxicity. The 10 day observation period was uneventful, and all animals survived to sacrifice with no noted ill-effects. Autopsy findings were that all viscera examined appeared normal.

Reference: Monsanto Y-73-168, Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

Type: LC_{50}
Species/strain: Rats
Exposure time: 6 Hours
Sex: Male
# of Animals: No data
Value: >400 mg/m3
Method: No data
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: no data
Remarks: RTECS inhalation LC50 citation
Reference: Kodak, May 21, 1971
Reliability: (4) Unassignable – data from a secondary literature source

5.1.3 ACUTE DERMAL TOXICITY

Type: LD_{50}
Species/strain: Rabbits, New Zealand Albino
Sex: Male and female
# of Animals: 5
Vehicle: None – undiluted
Doses: 1260, 2000, 3160, 5010 and 7940 mg/kg bw
Value: >3160 mg/kg bw
Method: Single Dermal Dose, Younger Laboratories Protocol, 1973
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 96%
Remarks: The undiluted test substance was applied to the shaved skin of male and female rabbits (1/sex/dose) for a period of 24 hours, followed by a 14 day recovery period. Males in this study weighed 2.4-2.6 kg, and females weighed 2.2-2.7 kg. Dosages were 1260, 2000, 3160, 5010 or 7940 mg/kg. The test material was held in place by means of an occlusive wrap of latex rubber and secured by bandaging and elastic tape. The occlusive wrap was removed after 24 hours and the excess material was wiped from the test animal. Clinical signs of toxicity were reduced appetite and activity – three to seven days in survivors – followed by increasing weakness, collapse and death. Deaths occurred in 2-3 days. Gross autopsy findings on decedents included lung hyperemia, liver discoloration, enlarged gall bladder and gastrointestinal inflammation. Survivors were sacrificed following the recovery period. All viscera appeared normal on all but two animals, which exhibited a slight discoloration of both liver and kidneys.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
1260	-	0/1	0/1
2000	0/1	-	0/1

3160	-	0/1	0/1
5010	1/1	-	1/1
7940	-	1/1	1/1

Reference: Monsanto Y-73-168, Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

Species/Strain: Rabbits, New Zealand Albino
Sex: Male and female
# of Animals: 6
Vehicle: None - undiluted
Value: 0.0/8.0
Results: Not Irritating
Classification: Non-Irritating
Exposure Time: 24 Hours
Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 96%
Remarks: 0.5 ml of the undiluted test substance was applied to the shaved dorsal areas of six albino rabbits. The test material was applied to the skin under 1" square gauze patches and held in contact with the skin by means of an occlusive wrap of latex rubber secured by bandaging and elastic tape. The occlusive wrap and gauze patches were removed after 24 hours.
Dermal irritation was scored by the Draize Method, and results were recorded 24, 48, 72 and 168 hours after topical application. The Primary Irritation Index was calculated by averaging the mean scores at 24 and 72 hours. The Primary Irritation Index was found to be 0.0 on a scale of 0.0-8.0. A slight defatting effect was noted, with skin flaking off in 7-10 days. There was no injury noted in depth.
Reference: Monsanto Y-73-168, Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

.

5.2.2 EYE IRRITATION/CORROSION

Species/strain: Rabbits, New Zealand Albino
Sex: Male and female
# of Animals: 6
Vehicle: None - undiluted
Value: 8.5/110.0
Results: Slightly irritating
Classification: Non-irritating
Exposure Time: 24 Hours
Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP: No data
Test substance: As prescribed in 1.1-1.4, purity: 96%
Remarks: 0.1 ml of the undiluted test substance was applied to one eye of six albino rabbits. The other eye was not treated and served as a control. The cornea, iris and conjuntivea were examined immediately after treatment, and then at intervals of 1 hour, and

at 24, 48, 72 and 168 hours.
The Draize Method was used for scoring eye irritation. Immediate findings: slight discomfort.
Immediate: slight discomfort
At 1 hour: slight erythema, very slight edema, copious discharge
At 24 hours: slight erythema, very slight edema, copious discharge
At 48 hours: slight erythema, very slight edema, moderate discharge
At 72 hours: slight erythema, very slight edema in two animals, slight to moderate discharge.
At 168 hours: all animals scored "0"
The average Draize score for 24, 48 and 72 hours was calculated for each animal and then averaged over the six animals. The average Draize score was 8.5 on a scale from 0-110.

Reference: Monsanto Y-73-168, Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.3 SKIN SENSITISATION

Type: Repeated Insult Patch Testing
Species/strain: Humans
Method: Modified Schwartz Method and Shelanski Method
Test substance: Other: Compounded rubber stocks w/test substance
GLP: No data
Results: Several studies were run using human volunteers to determine the potential of the test substance to cause allergic skin reactions from compounded rubber
stocks.
Loading of the test article was from 0.5 to 3 phr (parts per hundred rubber) in
a typical B-1 Masterbatch. Some study results indicated that the test article caused no primary irritation and no allergic response, while other study results were positive for sensitization.
Remarks: Differences in responses may be due to the presence of other chemicals in the B-1 masterbatch formulations.
Reference: Monsanto SH-61-17, Industrial Biology Labs, 1961
Monsanto SH-63-10, Industrial Biology Labs, 1963
Monsanto SH-64-4, Industrial Biology Labs, 1964
Monsanto SH-64-5, Industrial Biology Labs, 1964
Monsanto SH-73-12, Industrial Biology Labs, 1973
Reliability: (2) Valid with restrictions – mixture of chemicals

*5.4 REPEATED DOSE TOXICITY

Species/strain: Rats, Sprague-Dawley CD
Sex: Male/Female
# of animals: 60 (30 male, 30 female)
Route of Administration: Oral/Dietary
Exposure period: 30 days
Frequency of treatment: Daily
Post exposure observation period: None
Dose: 0, 100, 300, 500, 1000 and 2000 ppm
Control group: Yes
Concurrent no treatment

NOEL: 100 ppm (males)
300 ppm (females)
LOEL: Not determined
Results: In a 30-day range-finding study that preceded a 90-day study, the test substance was administered orally, via dietary admixture, to groups six-week old CD male and female rats (5/sex/group). Control animals received the standard laboratory diet. Physical observations, body weight and food consumption measurements were performed on all animals pretest and at selected intervals during the study. Hematology and chemistry determinations were performed on all animals at study termination. There were no mortalities during the course of the study. After four weeks of treatment, all animals were sacrificed, selected organs were weighed, and organ/body weight ratios were calculated. Complete postmortem examinations were conducted on all animals. Statistical evaluations included mean body weight, mean food consumption, mean clinical laboratory values, mean terminal organ/body weight and organ/body weight ratios via the appropriate one-way analysis of variance technique, followed by a multiple comparison procedure. Calculations for the statistical significance of differences were performed according to the method of Dunnett (1955). Differences from control in mean body weights were statistically significant at 500 ppm and 1000 ppm males and in 2000 ppm males and females. Differences from control in mean body weight/body weight gain suggested a treatment-related effect in males at dose levels at and above 300 ppm, and in females at and above 1000 ppm. Food consumption values in Week 1 were reduced for males at 500 ppm and above, and for females at 300 ppm and above. Food consumption at Weeks 3-4 was comparable to controls. Males and females at the two highest dose levels exhibited increased mean platelet counts following four weeks of treatment. Males in these groups also exhibited increased mean erythrocyte. The mean hematology values for males and females in all treatment groups were comparable to controls. Alterations in several clinical chemistry parameters were noted for higher dose levels. Mean terminal body weights were reduced at the two highest dose levels in females, and at the three highest dose levels in males. While several organs in treated males and females exhibited alterations in either mean absolute or relative weights, these changes were considered secondary effects and not indicative of significant organ toxicity. Gross pathological examination did not reveal any effects that were considered treatment-related.
Method: OECD Guidelines for Testing of Chemicals, Section 412, 1981
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99+% active
Reference: Monsanto BD-87-146 Bio/Dynamics Laboratories, 1987
Reliability: (1) Valid without restrictions

Species/strain: Rats, Sprague-Dawley CD
Sex: Male/Female
# of animals: 80 (40 males/40 females)
Route of Administration: Oral/Dietary
Exposure period: 90 days
Frequency of treatment: Daily
Post exposure observation period: None
Dose: Males: 0, 100, 250 and 500 ppm

	Females: 0, 250, 500 and 750 ppm
Control group:	Yes Concurrent no treatment
NOEL:	100 ppm for males Not established for females
LOEL:	Not Determined
Results:	The test substance was administered orally, via dietary admixture, to groups of 6-week old male and female CD rats (10/sex/group). Control animals received the standard laboratory diet. Physical observations, body weight and food consumption measurements were performed on all animals pretest and at selected intervals during the study. Hematology and chemistry determinations were performed on all animals at Months 1.5 and 3. There were no mortalities during the course of the study. After three months of treatment, all animals were sacrificed, selected organs were weighed, and organ/body and organ/brain weight ratios were calculated. Complete postmortem examinations were conducted on all animals. Histopathological evaluation of selected tissues was performed on all control and high-dose animals. The lungs, spleen, liver and kidneys were examined microscopically for all animals in all groups. Statistical evaluations included mean body weight, mean food consumption, mean clinical laboratory values, mean terminal organ/body weight, organ/body weight ratios and organ/brain weight ratios via the appropriate one-way analysis of variance technique, followed by a multiple comparison procedure. Calculations for the statistical significance of differences were performed according to the method of Dunnett (1955). Mean body weights and mean body weight gains were reduced in males at 250 and 500 ppm, and in all treated females. Overall, mean food consumption values for all treated groups were comparable to controls. Several clinical chemistry parameters exhibited statistically significant differences from control. Alkaline phosphatase was elevated in the 500 ppm males and 750 ppm females at Month 3. Mean serum glutamic oxaloacetic transaminase levels were significantly reduced in the 100, 250 and 500 ppm males at Month 1.5 but not at Month 3. Mean serum glutamic pyruvic transaminase was reduced in the 500 and 750 ppm females at Month 3. Several organs in the treated males and females exhibited alterations in mean absolute and/or relative (to body or brain) weight data. However, these alterations were generally consistent with the reductions noted in body weight data and were considered secondary effects which were not considered indicative of significant organ toxicity. There were no treatment-related findings noted in mortality, physical observations, opthalmoscopic, hematology, organ weight or gross and microscopic pathology.
Method:	OECD Guidelines for Testing of Chemicals, Section 453, 1981 and USEPA TSCA Section 4(a) Test Rules, 1982
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 99+% active
Reference:	Monsanto BD-87-147 Bio/Dynamics Laboratories, 1989
Reliability:	(1) Valid without restrictions

Species/strain:	Rats, Charles River Albino
Sex:	Male/Female
# of animals:	400 (200 males/200 females)
Route of Administration:	Oral/Dietary

Exposure period: 2 years
Frequency of treatment: Daily
Post exposure observation period: None
Dose: 0, 30, 100 or 300 ppm
Control group: Yes
Concurrent no treatment
NOEL: 30 ppm
LOEL: 100 ppm
Results: A two-year chronic oral toxicity study was conducted on groups of 400 CD Outbred rats (50/sex/dose) at dietary levels ranging from 0-300 ppm. Feeding of the test material began when the males were 28 days old, and the females 29 days old. Reductions in body weights and body weight gains were noted for males and females at the 300 ppm dose throughout the investigation. Body weights of females fed 100 ppm were reduced during the first 7 weeks and for 100 ppm males for the first 4 weeks. After those intervals, body weights compared favorably with controls. 30 ppm animals had body weights and weight gains that compared favorably with controls. Frequency and distribution of deaths during the investigation for all dose levels was similar to controls. Gross pathological examination of animals that died during the study did not reveal any relation between death and exposure to the test substance. No unusual behavioral reactions were noted in dosed animals during the course of the study. Results of hematologic studies conducted – total and differential leukocyte count, erythrocyte count, hemoglobin concentration, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration – were either similar to, or within the range of expected values for this strain of albino rats of this age and in this laboratory. Results of clinical blood chemistry studies (SGPT, BUN, SGOT, Fasting Blood Glucose Concentration, SAP) and of urinalyses (glucose, albumin, microscopic elements, pH and specific gravity) conducted showed similar results between control and test animals. Gross pathological examinations of animals sacrificed at 24 months revealed similar findings between test and control animals. Histopathological examinations of tissues and organs from the control and 300 ppm animals sacrificed at 24 months showed no treatment-related lesions. Microscopic examination of suspect neoplasms among all sacrificed animals and all animals that died during the study were conducted. No differences were noted between test and control rats as to the organ system involved, the type or the classification of neoplasms. The spectrum of neoplasms observed compared favorably to historical data at this laboratory for rats of this strain and age. At 17.5 months of testing, tetracycline HCl was added to the diets of all groups (30g/kg of diet) for a two-week period to treat a severe respiratory infection which caused an increase in mortality in both control and treated animals.
Method: 2-Year Chronic Oral Toxicity IBT Protocol # 622-05400B (1974)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99+% active
Reference: Monsanto BTL-74-27, Industrial Bio-Test Labs, 1978
Reliability: (1) Valid without restrictions

Species/strain: Rats
Sex: No data
# of animals: No data
Route of Administration: Inhalation

Exposure period: 22 weeks
Frequency of treatment: 4 hours/day
Post exposure observation period: No data
Dose: No data
Control group: No data
LOEL: TCLo = 100 mg/m3
Remarks: RTECS citation for 3081-14-9
Method: No data
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: not stated
Reference: TPKVAL, USSR, 1961
Reliability: (4) Not assignable – data from a secondary literature source

***5.5 GENETIC TOXICITY IN VITRO**

A. BACTERIAL TEST

Type: Ames Reverse Bacterial Mutation
System of testing: Salmonella typhimurium TA-98, TA-100, TA-1535, TA-1537
Concentration: 0.01, 0.04, 0.2, 1, 3, 10, 40 and 200 micrograms/plate (duplicate)
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 200 micrograms/plate
Without metabolic activation: 10 micrograms/plate
Precipitation conc: 1 microgram/plate
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Ames, B.N., McCann, J. and Yamaski, E. Methods for Detecting Carcinogens and Mutagens with the Salmonella Mammalian-Microsome Test. Mutat. Res. 31, 347-364, 1975
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99+% active
Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were 4-nitroquinoline-N-oxide (TA-98, TA-100), NaNO2 (TA-1535) and 9-aminoacridine (TA-1537). Chemicals used as positive controls for the activation assays were 2-acetylaminofluorene (TA-98), benzo(a)pyrene (TA-100), and 2-aminoanthracene (TA-1535, TA-1537). Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Positive control treatments produced the expected large increases in the frequency of histidine revertants. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.
Reference: Monsanto ML-85-242, Monsanto Environmental Health Labs, 1985
Reliability: (1) Valid without restriction

Type:	Bacterial Reverse Mutation - Ames
System of testing:	TA-98, TA-100, TA-1535, TA-1537, TA-1538
Concentration:	0.001, 0.01, 0.10, 1.00 or 5.00 ul/plate (duplicate)
Metabolic activation:	With and without
Results:	
Cytotoxicity conc:	With metabolic activation: 1.00 ul/plate Without metabolic activation: 5.00 ul/plate
Precipitation conc:	Not determined
Genotoxic effects:	
	With metabolic activation: Negative Without metabolic activation: Negative
Method:	Ames Plate Test (Overlay method) 1975
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 96%
Remarks:	The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were 10 ug/plate Methylnitrosoguanidine (MNNG), 100 ug/plate 2-nitrofluorene (NF) or 10 ug/plate Quinacrine mustard (QM). Positive controls used for the activation assays were 100 ug/plate 2-anthramine (ANTH), 100 ug/plate 2-Acetylaminofluorene (AAF) or 100 ug/plate 8-Aminoquinoline (AMQ). Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Statistical analysis was performed on plate incorporation assay results after transforming revertant/plate values as Log10 (revertants/plate). Analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present. Positive control treatments produced the expected large increases in the frequency of histidine revertants. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.
Reference:	Monsanto BIO-76-225, Litton Bionetics, 1976
Reliability:	(1) Valid without restriction

B. NON-BACTERIAL IN VITRO TEST

Type:	Mitotic Recombination Assay
System of testing:	Saccharomyces cerevisiae, D4
Concentration:	0.001, 0.01, 0.10, 1.00 or 5.00 ul/plate (duplicate)
Metabolic activation:	With and without
Results:	
Cytotoxicity conc:	With metabolic activation: 5.0 ul/plate Without metabolic activation. 0.1 ul/plate
Genotoxic effects:	
	With metabolic activation: Negative

Without metabolic activation: Negative

Method: Ames Mutagenicity Plate Test (Overlay Method) 1975

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 96%

Remarks: The test compound was evaluated for genetic activity with and without the addition of mammalian metabolic activation preparations. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. The chemical used as the positive control for the non-activation assay was methylnitrosoguanidine (MNNG) at 10 ug/plate. The positive control chemical used for the activation assay was DMNA at 100 micromoles/plate. Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Statistical analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: Monsanto BIO-76-225, Litton Bionetics, 1976

Reliability: (1) Valid without restriction

Type: Mammalian Cell Gene Forward Mutation Assay

System of testing: L5178Y Mouse Lymphoma cells

Concentration: 0.002, 0.004, 0.008, 0.016 (triplicate, without activation)
0.002, 0.004, 0.008, 0.016, 0.032 (triplicate, with activation)

Metabolic activation: With and without

Results:

Cytotoxicity conc: With metabolic activation: 0.032 ug/ml
Without metabolic activation: 0.016 ug/ml

Precipitation conc: Not determined

Genotoxic effects:

With metabolic activation: Negative
Without metabolic activation: Negative

Method: Clive and Spector, Mutation Research 31:17-29 (1975)

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity 96%

Remarks: The test article was evaluated for specific locus forward mutation in the L5178Y Thymidine Kinase (TK) mouse lymphoma cell assay. The cells used are heterozygous for a specific autosomal mutation at the TK locus and are BUdR sensitive. Scoring for mutation was based on selecting cells that have undergone forward mutation from a TK+/- to a TK-/- genotype by cloning them in soft agar with BUdR. Stock solutions were prepared in DMSO. DMSO was used as the negative control. The activation system was mouse liver S-9 mix. Ethylmethanesulfonate (EMS) at 0.5 ul/ml was used as the positive control without activation and Dimethylnitrosamine (DMN) at 0.3 ul/ml was used as the positive control with activation. The reference mutagens and induced mutation frequencies within the expected range.
The test article did not induce mutagenesis in either assay.

Conc. Mutant clones Viable clones Mutant frequency x10E-4

Non-Activation				
Solvent Control	---	34.0	122.0	0.2787
EMS	0.50	374.0	37.0	10.1081
Test Compound	0.25	19.0	106.0	0.1792
	0.50	20.0	109.0	0.1835
	1.00	27.0	142.0	0.1901
	2.00	19.0	123.0	0.1545
	4.00	27.0	110.0	0.2455
	8.00	Toxic		
Activation				
Solvent Control	---	64.0	170.0	0.4765
DMN	0.30	227.0	40.0	5.6750
Test Compound	1.00	43.0	141.0	0.3050
	2.00	30.0	171.0	0.1754
	4.00	18.0	107.0	0.1682
	8.00	35.0	89.0	0.3933
	16.00	21.0	150.0	0.1400
	32.00	Toxic		

Reference: Monsanto BIO-76-246 Litton Bionetics, 1976
Reliability: (2) Valid without restrictions

Type: *In vitro* Unscheduled DNA Synthesis (UDS)
System of testing: Primary rat hepatocyte cultures (Fischer-344 strain)
Concentration: 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, 50, 100, 500, 1000 ug/ml
Metabolic activation: With and without
Results:
Cytotoxicity conc: Preliminary Assay: 50 ug/ml
Replicate Assay: 5 ug/ml
Precipitation conc: Separation (two layers) at 1000 ug/ml
Genotoxic effects: Negative
Method: Williams, G.M., Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures, Cancer Research 37, pp. 1845-1851 (1977)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99+% active
Remarks: Acetone (1%) used as solvent and diluent. Primary rat liver cell cultures derived from the livers of two adult male rats weighing 248 and 284 grams (both 13 weeks old) were used for the preliminary and replicate experiments, respectively. Three controls were incorporated into each UDS assay: a positive control, a negative (solvent) control, and an untreated medium control. The positive control was 2-Acetylaminofluorene (2-AAF), the solvent control was acetone in the preliminary assay and in the replicate assay. The percentage of cells in repair was calculated as the percentage of cells with at least 5 net grains/nucleus. 150 cells were scored for each concentration reported for each experiment. Cytoxicity was observed at 50, 100 and 500 ug/ml in the preliminary experiment, and at 5, 10 and 20 ug/ml in the replicate experiment. Extreme separation of the test compound from the culture medium was evident at 1000 ug/ml in the preliminary experiment. The test compound was not completely miscible with the culture medium at concentrations above 20 ug/ml. UDS was measured at concentrations of the test compound between 0.01 and 1000 ug/ml in the preliminary experiment, and between 0.01 and 20 ug/ml in the replicate

experiment. All collection of data and pooling of slides were done via programs in the VAX 11/782 computer. The net grain counts were negative at each concentration of the test compound, in the solvent control and in the medium control, in contrast to the strong positive response produced by the positive control 2-AAF in both experiments (35.7 net grains/nucleus). These results indicate that the test compound is not a genotoxic agent under the conditions of the *in vitro* rat hepatocyte DNA repair assay.

Treatment	Conc.	NG	SE	Median	%IR
Control/medium	---	-19.1	4.2	-18.7	3
Control/solvent	1%	-16.3	0.5	-14.6	2
2-AAF ug/ml	0.5	35.7	1.4	35.2	93
Test Cpd. ug/ml	0.01	-20.9	1.9	-18.7	1
	0.05	-12.5	2.7	-12.1	1
	0.10	-12.2	1.2	-12.1	1
	0.50	-17.1	2.9	-16.5	1
	1.00	-15.9	0.6	-14.6	1
	5.00	Toxic			

Reference: Monsanto SR-85-250, SRI International, 1986
Reliability: (1) Valid without restriction

Type: CHO/HGPRT Forward Gene Mutation Assay
System of testing: CHO Cells, clone K1-BH4
Concentration:
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation:
Without metabolic activation:
Precipitation conc:
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: CHO/HGPRT Mutation Assay (1979) Hsie, et.al.
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99%
Remarks: The mutagenic potential of the test substance was evaluated in CHO cells for ability to induce forward mutation at the HGPRT gene locus. A range-finding cytotoxicity study preceded a dose-response mutagenicity experiment using different levels of Arochlor1254 rat liver homogenate (S9) concentrations, followed by a confirmatory dose-response mutagenicity experiment. The compound was tested at S9 concentrations up to a cytotoxic dose of 30 ug/ml. Solutions of the test compound were prepared using DMSO as the solvent on the day of treatment. Positive controls used were benzo(a)pyrene and ethyl methane sulfonate for the activation and non-activation assays, respectively. The subclone K1BH4 of CHO cells was obtained from Dr. Hsie of Oak Ridge National Laboratories. CHO cells were plated the day before treatment. Statistical analysis was according to the methos of Snee and Irr (1981) designed specifically for the CHO/GHPRT mutation assay. Student's t-test was used to compare treatment data to control data. The Snee and Irr analysis also allowed the determination of dose-response relationship as linear, quadratic, or higher

order. A computer program obtained from Joe Irr was used. No statistically significant mutagenicity was observed in the two separate experiments. The positive controls yielded the expected positive responses in mutagenicity, indicating the adequacy of the experimental conditions. Therefore, the test substance was not considered to be mutagenic in CHO cells under the experimental conditions.

Reference: Monsanto ML-85-222, Environmental Health Laboratory, 1986
Reliability: (1) Valid without restriction

*** 5.6 GENETIC TOXICITY IN VIVO**

5.7 CARCINOGENICITY

***5.8 TOXICITY TO REPRODUCTION**

Type: Fertility
Other: Three Generation Study
Species/strain: Rats, Charles River Albino
Sex: Male/Female
Route of Administration: Oral/Dietary
Exposure period: Premating, throughout mating, gestation and lactation
Frequency of treatment: Daily
Post exposure observation period: Not Determined
Premating exposure period: F0 – 14 wks (males)
F1-- 14 wks (males)
F2 – 18 wks (males)
F0 – 14 wks (females)
F1 – 14 wks (females)
F2 – 18 wks (females)
Duration of the test: F0 – 23 wks
F1 – 23 wks
F2 – 26 wks
Doses: 0, 30, 100 or 300 ppm
Control group: Yes
Concurrent no treatment
NOEL Parental: 30 ppm (based on reduced body weight gain)
NOEL F1 Offspring: 30 ppm (based on reduced pup survival)
NOEL F2 Offspring: 30 ppm (based on reduced pup survival)
Results: The test compound was administered to three successive generations of rats at dose levels of 0, 30, 100 or 300 ppm. Dose levels were selected on the basis of results from a previous 2-year chronic oral feeding study. No adverse effects on mating or fertility indices were noted in any of the treated animals. Reduced survival of offspring was observed in the mid- to high-dose groups. Evidence of parental toxicity was also present as indicated by reduced body weights of the mid-to high-dose animals.
General parental toxicity: Reduced body weights and mean body weight gains were noted for the 100 and 300 ppm males and females. No other treatment-related effects were evident in results of clinical blood chemistry studies and urinalyses results between the control groups and the treated animals.
Toxicity to offspring: A small but statistically significant reduction in the survival rates of pups was noted in the 100 ppm and 300 ppm groups.

Method: 3-Generation Reproductive Toxicity IBT Protocol # 622-05400C (1974)
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 99+% active
Remarks: Protocol similar to Monsanto BTL-74-27, Industrial Bio-Test Labs, 1978
Reference: Monsanto BTL-76-145, Industrial Bio-Test Labs, 1976
Reliability: (1) Valid without restriction

***5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY**

Species/strain: Rats, Charles River CD Albino
Sex: Female
Route of Administration: Oral gavage
Duration of the test: 25 days from mating to last C-section
Exposure period: Day 6-15 of gestation
Frequency of treatment: Daily, as a single oral dose at a volume of 5 ml/kg
Doses: 0, 25, 75 or 150 mg/kg/day
Control group: Yes
Concurrent vehicle
NOEL Maternal Toxicity: 25 mg/kg/day
NOEL teratogenicity: 150 mg/kg/day
Results: Groups of 25 mated CD rats were assigned to one control group and three treatment groups to determine the teratogenic potential of the test substance. Dosage levels of 25, 75 and 150 mg/kg/day were administered orally by gavage as a single daily dose on Days 6-15 of gestation. The control group received the corn oil vehicle only. Cesarean sections were performed on all surviving females on gestation Day 20, and the fetuses removed for teratologic evaluation.
Maternal general toxicity: Toxicity in the dams was apparent at the 75 and 150 mg/kg/day dosage levels. Parameters adversely affected were maternal survival, appearance, behavior and body weight gain. Four of the 150 mg/kg/day females and one 75 mg/kg/day female died between gestation Days 16-17. Control animals and the low dose group had 100% survival. Antemortem abnormalities in the decedents included dried blood around and/or expelled from the vaginal orifice, blood under the cage, stained, wet or matted coat, hypothermia and ptyalism. There were no treatment-related gross internal lesions evident. No effect on Cesarean section observations was noted in the dams at any dosage level.
Pregnancy/litter data: No obvious differences were noted between the treated groups and the control group.
Foetal data: Malformations that were observed in the treated groups occurred
in low incidence and were not considered treatment-related. One high-dose fetus had anophthalmia, one mid-dose and two control group fetuses had microphthalmia, and another mid-dose fetus had ectopia cordia and sternoschisis. There were no adverse effects on the fetal parameters examined (survival, growth, morphological development) at dose levels at or below 150 mg/kg/day.
Method: OECD Guidelines for Testing of Chemicals No. 414 “Teratogenicity” 1981, and TSCA Health Effects Guidelines “Teratogenicity Study” 1982
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99+% active
Remarks: Based on the results, the test article did not induce developmental toxicity In the offspring of Charles Rived CD rats under the test conditions.

Reference: Monsanto IR-85-290 International Research and Development, 1986
Reliability: (1) Valid without restrictions

5.10 OTHER RELEVANT INFORMATION

*** 5.11 EXPERIENCE WITH HUMAN EXPOSURE**

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I U C L I D

D a t a S e t

Existing Chemical	ID: 68953-84-4
CAS No.	68953-84-4
EINECS Name	1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs.
EINECS No.	273-227-8

Producer Related Part	
Company:	ACC Rubber and Plastics Additives Panel
Creation date:	31-July-2000
Substance Related Part	
Company:	ACC Rubber and Plastics Additives Panel
Creation date:	31-July-2000

Printing date:	22-JAN-2003
Revision date:	
Date of last Update:	22-Jan-2003

Number of Pages:	51

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Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

Date: 22-Jan-2003

1. General Information ID: 68953-84-4

1.1 General Substance Information

Substance Type:
Physical Status: solid
Purity: 90 - 95 wt. %
Result: Molecular Weight: 274 (avg.)

1.1.1 Spectra

1.2 Synonyms

1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs.

Accinox 100

Blend of phenyl and tolyl p-phenylenediamines

DAPD

Mixed diaryl-p-phenylenediamines

Mixed di-aryl-p-phenylenediamines

Diaryl-p-phenylenediamines

Naugard 496

Vulkanox 3100

Wingstay 100

Polystay 100

WTR Number 4a

Nailax (Nailax B)

Remark: Complex reaction product containing;
N,N'-di(o-tolyl)-p-phenylenediamine;
N.N'-Diphenyl-p-phenylenediamine; and
N-Phenyl-N'-(o-tolyl)-p-phenylenediamine

Date: 22-Jan-2003

1. General Information ID: 68953-84-4

1.3 Impurities

CAS Number: 95-53-4
EINECS Number: 202-429-0
Chemical Name: o-Toluidine
Contents: < 0.1 wt %

CAS Number: 62-53-3
EINECS Number: 200-539-3
Chemical Name: aniline
Contents: < 0.1 wt %

CAS Number: 552-82-9
EINECS Number: 209-023-2
Chemical Name: Methyldiphenylamine
Contents: < 0.1 wt %

CAS Number: 122-39-4
EINECS Number: 204-539-4
Chemical Name: Diphenylamine
Contents: 1 - 5 wt %

1.4 Additives

2.1 Melting Point

Value: 90 - 105 degree C
Decomposition: ambiguous
Method: other: ASTM D-1519
Year: 1993
GLP: no
Reliability: (2) valid with restrictions
Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.
31-JUL-2000 (35)

2.2 Boiling Point

2.3 Density

Type:
Value:
Method: Other: ASTM D-891
Result: Specific Gravity: 1.18
Reliability: 2) valid with restrictions
Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.
31-Jul-2000 (34)

2.4 Vapour Pressure

2.5 Partition Coefficient

log Pow: 3.4 - 4.3
Method: OECD Guide-line 117 "Partition Coefficient n-Octanol/Water), HPLC Method"
Year: 1995
GLP: yes
Remark: The product exhibits much lower values than DDT (6.2) which provides a benchmark for highly bioaccumulative chemicals. The test substance contains 3 major components.
Result: # Methyl Groups -0 log Pow 3.37
Methyl Groups -1 log Pow 3.82
Methyl Groups -2 log Pow 4.28

The major components of the test substance displayed partition coefficients between 3.4 and 4.3.[as prescribed by 1.1-1.4 (Wingstay 100, mixed diaryl-p-phenylenediamines)]
Reliability: (1) valid without restriction
01-AUG-2000 (29)

log Pow: > 3.7 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: for N,N'-Diphenyl-p-phenylenediamine
Reliability: (1) valid without restriction
20-FEB-2001 (9)

log Pow: > 4.3 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: For N-phenyl-N'-(o-tolyl)-p-phenylenediamine
Reliability: (1) valid without restriction
31-JUL-2000 (9)

log Pow: > 4.6 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: For N,N'-Di(o-tolyl)-p-phenylenediamine
Reliability: (1) valid without restriction
20-FEB-2001 (9)

3.1.1 Photodegradation

3.1.2 Stability in Water

Type:
Method:
Year: 1994 GLP: yes
Test substance: as prescribed by 1.1 - 1.4 (Mixed diaryl-p-phenylenediamines)
Remark: See Biodegradation Studies
Reliability: (1) valid without restriction
31-JUL-2000 (23)

3.3.1 Transport between Environmental Compartments

3.5 Biodegradation

Type: anaerobic
Inoculum: activated sludge, domestic
Concentration: 100 mg/l related to Test substance
Degradation: .64 % after 28 day
Result: other: not readily biodegradable
Method: OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year: 1994 GLP: yes
Test substance: as prescribed by 1.1 - 1.4 (Mixed diaryl-p-phenylenediamines)
Reliability: (1) valid without restriction
31-JUL-2000 (23)

Type: anaerobic
Inoculum: activated sludge
Degradation: 0 % after 28 day
Method: other: OECD 301 Manometric Respirometry, modified according to EEC Round Robin Test "Assessment of Respirometry" DGX 1/283/82
Rev. 6, EEC Directive 79/831, Annex V, Part C
Year: 1990 GLP: yes
Test substance: as prescribed by 1.1 - 1.4 (Mixed diaryl-p-phenylenediamines)

Reliability: (1) valid without restriction
31-JUL-2000 (6)

3.6 BOD5, COD or BOD5/COD Ratio

Method: other: unknown
Method: other: unknown

Result: ThOD: 3056 mg/g
Reliability: (4) not assignable

(6)

Method: other: unknown
Method: other: unknown

Result: ThOD: 2.555 mg/mg
Reliability: (4) not assignable

(23)

3.7 Bioaccumulation

Species:	Cyprinus carpio (Fish, fresh water)
Exposure period:	56 day
Concentration:	.05 mg/l
BCF:	< 5000
Elimination:	
Method:	other: MITI Method for Testing the Degree of Accumulation of Chemical Substances in Fish Bodies
Year:	1998 GLP: yes
Test substance:	as prescribed by 1.1 - 1.4 (Wingstay 100, mixed diaryl-p-Phenylenediamines)
Method:	The test substance had an assumed purity of 100%. A pilot toxicity test used orange-red killifish (Oryzias latipes) (10 fish per level) exposed the test substance for 48-hours in a semi-static system. Stock solutions were prepared by dissolving the test substance and HCO-40 (hydrogenated castor oil; 20 times the amount of the test substance) in tetrahydrofuran. Following evaporation of the tetrahydrofuran,ion-exchanged water was added to the mixture to prepare a 500 mg/L stock solution of the test substance. Carp (Cyprinus carpio)was used as the test species for the Bioconcentration study. Based on the 48-hours toxicity results and analytical detection, the test concentrations used were Level 1 (high exposure level)-0.05 mg/L and Level 2 (low exposure level)-0.005 mg/L. The test tanks were 100 L glass tanks. The test solution was entered into mix tanks at a flow rate of two(2) mL/minute for the stock solution and 1600 mL/minute for the dilution water. For controls, HCO-40 was dissolved with ion-exchanged water to give a 800 mg/L solution. The duration of exposure was for 8-weeks. Dissolved oxygen in the test tanks was measured twice a week. The concentrations of the test substance in water for both Levels were analyzed twice per week throughout the study. The concentrations of the test substance in fish at both Levels were analyzed during Week -1, -2, -4, -6 and –8 {two (2) fish per week}. Control fish were analyzed at the initiation {two (2) fish} and at termination {two (2) fish} of exposure. Additional fish were subjected to analysis on Days -1, -5, and -8 following cessation of exposure on Study Day-56 to assess depuration of test substance from fish tissues. All tissue and test water samples were analyzed using high performance liquid chromatography (HPLC).

Water levels were analyzed by loading large volumes on C18 Sep Pak mini-column, which was then eluted from column with Acetonitrile containing 0.1% Formic acid. The final volume of eluate was 5 mL. Test fish were analyzed by measuring weights, body lengths, chopping into pieces, and extracting with Acetonitrile. The mixture was centrifuged {7000xg. Five (5) minutes} and the supernatant was filtered with absorbent cotton to a volume of 100 mL. Two (2) separate samples were analyzed to assess Diphenylamine (DPA) and Diaryl p-phenylenediamine (diaryl-PPD) components (87% of complex)and to assess higher molecular weight components (13 % of complex). All recovery and blank tests were carried out in duplicate.

Remark: For DPA and DPPD compounds, methyl substitution increased bioaccumulation in carp, consistent with increasing log Po values. Substantial variation occurred at each time point due to use of data from a maximum of 2 fish. While this project provided substantial data, further work was needed to calculate BCFs according to western (OECD) concepts, and to apply appropriate statistics to these data so as to provide basis for interpretation.

To address this issue, a project was conducted by McLaren Hart entitled "Statistical Calculations of Data from a Bioaccumulation Study with WINGSTAY 100 in Carp", November 25,1998. The analysis employed Monte Carlo methods; the maximum BCF value (Pk 5) was 6600, and depuration data confirmed the attainment of tissue steady state levels of WINGSTAY 100 components within 3 weeks. Depuration was confirmed to be < 5 days for all components.
Orange-red killifish (Oryzias latipes) were used in the pilot toxicity test.

Result: Bioconcentration Test: The laboratory had difficulty maintaining nominal concentrations, possibly due to rapid uptake and metabolism by the fish and partioning to tank surfaces. The test concentrations ranged from 60 to 100% of the nominal values. The Bioconcentration Factors (BCFs) were calculated from individual data for fish at each time point and by using time-weighted averages for water concentrations. Since the test substance was a complex reaction product with numerous peaks, there was a high degree of variability in the fish data resulting in a large range of BCF values (20-221 for Peak 1; 128-659 for Peak 2; 269-2460 for Peak 3; 776-3640 for Peak 4; 2980-11300 for Peak 5). Depuration results for components indicated half-lives were below five (5) days for all components with the exception to one (1) estimate of 44-days for Peak 5. This inconsistent value appears to be suspect since it is much higher than the value of 4.7 days that was obtained for the same Peak in the other concentration. Also, the value is inconsistent with the trend Observed for half-lives for Peaks 1 through 4.

Bioconcentration Factors (BCFs) were calculated by using individual data points, including those prior to reaching steady-state. Estimates of steady-state through the use of Monte Carlo modeling improved the estimations of the BCFs. The bioaccumulation data and depuration data can be used together in performing analyses, particularity when the collected bioaccumulation data contained information on half-lifes(i.e., time to reach steady-state). The Monte Carlo "best estimates" for BCFs were < 5000 for all components except Peak 5 which had a BCF of approximately 7000.
Pilot Toxicity Test: The 48-hour LC50 result for the test substance in orange-red killifish was 17.2 mg/L. **Please note**: this concentration was achieved only through the use of a surfactant {Hydrogenated Castor Oil (HCO-40)}, and is far above the test substance solubility in water (approximately 2 mg/L). MITI guidelines recommend levels for Bioaccumulation testing to be at 1/1000 and 1/10,000 of the LC50 value. The lower value would have been below the quantitation range; thus, 0.005 and 0.05 mg/L were chosen.

Test condition: Two (2) test concentrations were used: Level 1 (high exposure level)-0.05 mg/L and Level 2 (low exposure level)-0.005 mg/L

Reliability: (1) valid without restriction

(10)

Date: 22-Jan-2003
4. Ecotoxicity ID: 68953-84-4

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type:	flow through
Species:	Cyprinus carpio (Fish, fresh water)
Exposure period:	14 day
Unit:	mg/l Analytical monitoring: yes
NOEC:	.28
LC50:	.43
Method:	OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"
Year:	1996 GLP: yes
Test substance:	Wingstay 100 (mixed di-aryl-p-phenylenediamines)
Method:	Test water was generated by adding the test substance in acetone to a larger volume of water which was stirred, allowed to settle, and then siphoned to a stock solution holding tank. This stock solution was then metered into exposure tanks for the fish experiments. A range-finding trial exposed carp to nominal levels of 2.5, 5, 10, and 25 mg/L (ppm) of the test substance. Survival rates were up to 80% within the first 48 hours for the three (3) highest dose levels and the 2.5 mg/L induced no mortality in the first 48 hours although 90% deaths were seen through Day six (6). In the definitive phase, duplicate test tanks contained 10 carp each and the test substance nominal concentrations of 0, 0.1, 0.23, 0.51, 1.1, and 2.5 mg/L (ppm). Chemical analysis (HPLC) of the test substance in the test tanks on Days -0, -3, -7, and -14 showed that mean concentrations for the 14-day test period were 0.053, 0.12, 0.19, 0.28, and 0.67 mg/L (ppm). Fish densities were 0.35 g biomass/L flowing test solution per day. Tank volume turnover for the flow-through system was 6.5/day. Carp were monitored daily for mortality and signs of erratic swimming behavior for 14 days during exposure. Body weights and lengths were recorded for representative fish prior to study initiation, and on all test fish on Day 14. A LC50 value was then calculated.
Result:	Carp died only at the highest test substance concentration; 2/20 on Day-3, 7/20 on Day-7, and 20/20 by Day-14. Other findings at the 0.67 mg/L (ppm) level included darkened pigmentation on the fish (likely due to adsorption of the test chemical), lethargic swimming behavior, and loss of equilibrium. There were no test substance-related effects on body lengths or weights.
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (1) valid without restriction
20-FEB-2001 (30)

Date: 22-Jan-2003
4. Ecotoxicity ID: 68953-84-4

Type: flow through
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 14 day
Unit: mg/l Analytical monitoring: yes
NOEC: .14
LC50: .26
Method: OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"
Year: 1997 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Test water was generated by adding the test substance in acetone to a larger volume of water which was stirred, allowed to settle, and then siphoned to a stock solution holding tank. This stock solution was then metered into exposure tanks for fish experiments. A preliminary study in trout was performed using nominal concentrations of the test substance of 0.1, 0.23, 0.51, 1.1, and 2.5 mg/L. Mortality rates were 100% at the highest level by Day-3, and was 80% by Day-7 at 1.1 mg/L.

In the definitive phase, duplicate test tanks contained 10 trout each, Test substance nominal concentrations of 0, 0.094, 0.19, 0.38, 0.75, and 1.5 mg/L (ppm) were chosen. Chemical analysis (HPLC) of the test substance in the test tanks on Days -0, -7 and -14 showed that mean concentrations for the 14-day test period were 0.062, 0.093, 0.14, 0.35, and 0.66 mg/L (ppm). Fish densities were 0.079 g biomass/L flowing test solution per day. Tank volume turnover for the flow-through system was 6.5/day. Fish were monitored daily for mortality and signs of erratic swimming behavior for 14-days during exposure. Body weights and lengths were recorded for representative fish prior to study initiation, and on all test fish on Day-14. LC50 values were calculated for 96-hours and 14-days.

Result: Fish died only at 0.35 and 0.66 mg/L concentrations; 0/20 and 1/20 died by Day-2 and 1/20 and 19/20 by Day -4 , respectively. Further, 100 % of the high dose (0.66 mg/L) fish died by Day-5 and 17/20 of the 0.37 mg/L fish by Day-14. Other findings at the two highest levels included darkened pigmentation of the fish, lethargic swimming behavior, and loss of equilibrium. There were test substance-related effects on 14-day body lengths and weights in the 0.35 mg/L group. The calculated LC50 for the test substance in the study at 96-hours was 0.48 mg/L and 0.26 mg/L at 14-days. The No Observed Effect Concentration (NOEC) was 0.14 mg/L at 96-hours and 14-days.

Reliability: (1) valid without restriction
31-JUL-2000

(38)

4.2 Acute Toxicity to Aquatic Invertebrates

Species:	Daphnia magna (Crustacea)
Exposure period:	48 hour(s)
Unit:	mg/l Analytical monitoring: yes
NOEC:	.36
EC50:	1.8
Method:	OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year:	1996 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: A range-finding study used ten (10) 24-hour old daphnids exposed to nominal levels of 0, 13,22,36,60, and 100 mg/L of the test substance. Immobilization (15%) of the daphnids occurred at the highest level (100 mg/L). Sublethal lethargy was observed at all but the lowest test concentration (13 mg/L). Brown matter, apparently the test substance since brown precipitate was observed in the media, was observed to adhere to both surviving and non-surviving daphnids.

In the definitive phase, duplicate aquaria containing 10 daphnids each and test substance nominal concentrations of 0, 1.3, 2.2, 3.6, 6.0 and 10 mg/L (ppm) were prepared. Mean values for the test substance concentrations in the test media were determined by averaging chemical analyses (HLPC) of 0-hours and 48-hours.

Daphnia immobilization and aquaria observations were made at 24- and 48-hours following the study initiation. From these data, an Effective Concentration in one-half the organisims (EC50) and a No Observed Effect Concentration (NOEC) were estimated.

Result: Measured concentrations of the test substance ranged from 19 to 29% of nominal levels. At the highest concentration (1.8 mg/L), 25 % of the daphnids were immobilized at 48-hours of exposure. For the 0.68 and 1.1 mg/L groups, Five (5) % of the daphnids were immobile. No immobilization was observed at 0.20 and 0.36 mg/L exposures. Lethargic activity was not observed at any treatment level. Brown particulates, perhaps the test substance, were observed to adhere to the test daphnids, with some buoyed to the surface of the aquaria by this particulate material. The results indicated that the EC50 for the test substance was 1.8 mg/L. The No Observed Effect Concentration (NOEC) was shown to be 0.36 mg/L.

Reliability: (1) valid without restriction
31-JUL-2000 (28)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species:	Selenastrum capricornutum (Algae)
Endpoint:	biomass
Exposure period:	72 hour(s)
Unit:	µg/l Analytical monitoring: yes
NOEC:	4.3
EC10:	4.3
EC50:	18
Method:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year:	1996 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: A range-finding trial used nominal levels of 0, 1,10, 100, and 1000 ug/L (ppb) of the test substance and a solvent control in algae cultures (approximately 1x104 cells per flask). Following 72-hours incubation, algal cell densities were determined using a hemacytometer. Values were 127,76,109,69 and 1%, respectively, of the solvent control response. These values were used to set exposures for the definitive phase.

In the definitive phase, triplicate algal cultures were exposed to the test substance at nominal concentrations of 16, 31, 63,130, 250, and 500 ug/L (ppb). Cell densities were monitored at 24-, 48-, and 72-hours following study initiation. From these data, EC50 (50% decrease) values for Biomass (EbC50) and Growth Rate (ErC50) were calculated. Test substance concentrations in the test media were determined at 0- and 72-hours using HLPC. The mean concentrations were 7.5, 13, 14, 28, 50, and 79 ug/L (ppb).

Result: The inhibitions of algae Growth Rates for the test substance in the definitive 72-hour study were 0, 2, 15, 20, 32, and 38% (relative to pooled control values) for the measured test substance concentrations of 7.5, 13, 14, 28, 50, and 79 ug/L (ppb). Corresponding inhibitions of Biomass generation were 15, 41, 59, 63, 81, and 91%. Individual cell appearances were found microscopically to be normal for surviving cells except cellular bloating was noted at the highest exposure level. Calculations indicated that the ErC50 for the test substance was > 79 ug/L (ppb) while the EbC50 was 18 ug/L (ppb). The No Observed Effect Concentrations (NOECs) were assumed to be equivalent to EC10 values, and accordingly were EbC10 = 4.3 ug/L (ppb) and ErC10= 31 ug/L (ppb).

The EC50 values for the test substance ranged from 18 to > 79 ug/l (ppb) for Biomass increases and Growth Rates. The NOECs ranged from 4.3 to 31 ug/L (ppb) for these parameters.

Reliability: (1) valid without restriction
31-JUL-2000 (31)

Date: 22-Jan-2003
4. Ecotoxicity ID: 68953-84-4

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: µg/l Analytical monitoring: yes
NOEC: 31
EC10: 31
EC50: > 79
Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 1996 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: A range-finding trial used nominal levels of 0, 1,10, 100, and 1000 ug/L (ppb) of the test substance and a solvent control in algae cultures (approximately 1x104 cells per flask). Following 72-hours incubation, algal cell densities were determined using a hemacytometer. Values were 127,76,109,69 and 1%, respectively, of the solvent control response. These values were used to set exposures for the definitive phase.

In the definitive phase, triplicate algal cultures were exposed to the test substance at nominal concentrations of 16, 31, 63,130, 250, and 500 ug/L (ppb). Cell densities were monitored at 24-, 48-, and 72-hours following study initiation. From these data, EC50 (50% decrease) values for Biomass (EbC50) and Growth Rate (ErC50) were calculated. Test substance concentrations in the test media were determined at 0- and 72-hours using HLPC. The mean concentrations were 7.5, 13, 14, 28, 50, and 79 ug/L (ppb).

Result: The inhibitions of algae Growth Rates for the test substance in the definitive 72-hour study were 0, 2, 15, 20, 32, and 38% (relative to pooled control values) for the measured test substance concentrations of 7.5, 13, 14, 28, 50, and 79 ug/L (ppb). Corresponding inhibitions of Biomass generation were 15, 41, 59, 63, 81, and 91%. Individual cell appearances were found microscopically to be normal for surviving cells except cellular bloating was noted at the highest exposure level. Calculations indicated that the ErC50 for the test substance was > 79 ug/L (ppb) while the EbC50 was 18 ug/L (ppb). The No Observed Effect Concentrations (NOECs) were assumed to be equivalent to EC10 values, and accordingly were EbC10 = 4.3 ug/L (ppb) and ErC10= 31 ug/L (ppb).

The EC50 values for the test substance ranged from 18 to > 79 ug/l (ppb) for Biomass increases and Growth Rates. The NOECs ranged from 4.3 to 31 ug/L (ppb) for these parameters.

Reliability: (1) valid without restriction
31-JUL-2000 (31)

Date: 22-Jan-2003
4. Ecotoxicity ID: 68953-84-4

4.4 Toxicity to Microorganisms e.g. Bacteria

Type:	aquatic
Species:	activated sludge
Exposure period:	30 minute(s)
Unit:	mg/l Analytical monitoring: no
EC50:	> 10000
Method:	ISO 8192 "Test for inhibition of oxygen consumption by activated sludge"
Year:	1993 **GLP:** yes
Test substance:	as prescribed by 1.1 - 1.4
Reliability:	(1) valid without restriction

(6)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type:	LD50
Species:	rat
Strain:	
Sex:	no data
Number of Animals:	
Vehicle:	
Value:	> 2000 mg/kg bw
Method:	other: Directive 84/49/EEC, B.1
Year:	1990 GLP: yes
Test substance:	as prescribed by 1.1 - 1.4
Reliability:	(1) valid without restriction

01-AUG-2000 (7)

Type:	LD50
Species:	rat
Strain:	
Sex:	male/female
Number of Animals:	10
Vehicle:	other: corn oil
Value:	> 5000 mg/kg bw
Method:	other: US EPA 40CFR798.2650, Oral Toxicity-Limit Test
Year:	1993 GLP: yes
Test substance:	as prescribed by 1.1 - 1.4

Method: Five (5) male and five (5) female young adult rats (Sprague-Dawley) were administered a single dose of the test substance by gavage. The test substance was dispersed in corn oil (Sigma Chemical Company) and administered at a dosage of 5000 mg/kg. The animals were observed for clinical signs of toxicity at approximately 1-, 4- and 24-hours following administrations on the day of dosing and daily thereafter for 14-days. Body weights were recorded on Day-0, Day-7 and Day-14. All animals were subjected to a gross necropsy at study termination.

Result: One (1) animal died during the 14-day observation period. Clinical signs observed included decreased activity, decreased muscle tone, and diarrhea. No significant impairment on body weight gains were noted in either the male or female rats. Necropsy of the animal that died during the study revealed discolored kidneys, spleen, and liver. No visible lesions were observed in any of the animals at terminal necropsy. The estimated acute oral LD50 (combined sexes) for the test substance was determined to be > 5000 mg/kg.

Reliability: (1) valid without restriction

01-AUG-2000 (20)

Date: 22-Jan-2003
5. Toxicity ID: 68953-84-4

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: > 4000 mg/kg bw
Method: other
Year: 1959 GLP: no
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Result: No animals died at the single high dose of 4000 mg/kg.

Reliability: (4) not assignable
01-AUG-2000 (39)

5.1.2 Acute Inhalation Toxicity

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain:
Sex: male/female
Number of
Animals: 10
Vehicle: other
Value: > 2000 mg/kg bw
Method: OECD Guide-line 402 "Acute dermal Toxicity"
Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Albino rabbits (five males and five females) were shaved in the caudal portion of the animals' trunks. One (1) day later, a 2000 mg/kg dose of 40 mesh test substance (obtained by grinding in mortar/pestle) was placed onto the skin sites (approximately 10% of the body surface areas). The application sites were then covered with gauze, plastic, and elastic wraps and finally secured with non-irritating tape. After 24-hours of skin contact to the exposure areas, the gauze patches were removed and adhering test substance removed with moistened gauze. Skin test sites were scored for signs of erythema (redness) and edema (swelling) according to Draize procedures from Day-1 to Day-14 following cessation of exposures. Animals were observed for adverse clinical signs, mortality, and body weights (Day-0, Day-7, and Day-14). Necropsies were performed on the final day of observations (Day-14).

Remark: A limit test

Result: The test substance induced no deaths or apparent adverse clinical signs. Mild irritation (Grades 1,2 erythema; Grade 1 edema) was seen at skin sites of treated rabbits for periods ranging from Day-1 to Day-10. Staining of skin was noted due to the dark color of the test substance. A body weight decrease was seen in one (1) of the ten (10) rabbits between Day-7 and Day-14. No compound-related non-dermal findings were observed in the study. No mortality or adverse clinical/necropsy changes were observed associated with the test substance. The dermal LD50 for the test substance was shown to be > 2000 mg/kg.

Reliability: (1) valid without restriction
01-AUG-2000 (27)

5.2.1 Skin Irritation

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classification: not irritating
Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1991 GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Exposure period: 4 hours
Reliability: (2) valid with restrictions
(8)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classification: not irritating
Method: other: A 20% suspension of the material was applied to the shaved test site of six albino rabbits.
Year: 1959 GLP: no
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)
Reliability: (4) not assignable
(39)

Date: 22-Jan-2003
5. Toxicity ID: 68953-84-4

Species: rabbit
Concentration: undiluted

Exposure: Occlusive
Exposure Time: 4 hour(s)

Number of Animals: 6
PDII: .46
Result: slightly irritating
EC classification: not irritating
Method: Draize Test
Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Albino rabbits (six females) were shaved in the caudal Portion of the animals' trunks. One (1) day later, 0.5 grams of 40 mesh test substance (obtained by grinding in mortar/pestle) was placed on a one (1) inch squares of cotton gauze, moistened with water, applied to the skin sites, and secured with non-irritating tape. After 4-hours of skin contact exposures, the gauze patches were removed and adhering test substance removed with moistened gauze. Skin test sites were scored for signs of erythema (redness) and edema (swelling) according to Draize procedures at 1-, 24-, 48-, and 72-hours following cessation of exposures. Gross necropsies were performed on the animals following final scoring of the skin sites.

Result: The test substance induced no deaths or apparent adverse clinical or postmortem signs. Slight erythema (redness) was seen at skin sites of five (5) out of six (6) treated rabbits for maximum periods ranging from 1- to 48-hours. Staining of skin was noted due to the dark color of the test substance. The calculated irritation score was 0.46. The test results indicate an irritation rating as a "SLIGHT IRRITANT" and as a "NON-CORROSIVE".

Reliability: (1) valid without restriction

(26)

5.2.2 Eye Irritation

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: not irritating
EC classification: not irritating
Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1991 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Remark: Exposure period: 24 hours

Reliability: 2) valid with restrictions
(8)

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: 72 hour(s)
Comment: rinsed after (see exposure time)
Number of Animals: 9
Result: slightly irritating
EC classification: irritating
Method: Draize Test
Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: The eyes of albino rabbits (9-both genders) were examined using fluorescein dye and UV light for evidence of corneal damage and dye retention. Animals found to be acceptable received approximately 0.06 grams (0.1 mL) of 40 mesh test substance (obtained by grinding in mortar/pestle) applications to the right eyes. After 30-seconds of eye contact to the test substance, a water rinse was applied to three (3) of the nine (9) rabbits in an attempt to minimize chemical irritation. Left eyes were untreated and served as control sites. Eyes were assessed for signs of gross corneal, iridal, or conjunctival injury according to Draize procedures at 1-, 24-, 48-, and 72-hours (7-days for one (1) rabbit with eye damage at 72-hours). Fluorescein dye exams were conducted at 24-hours.

Result: The test substance induced no adverse clinical signs. No corneal damage was induced in any of the unrinsed rabbits although one (1) out of six (6) rabbits exhibited dye retention judged to be non-chemically related. Conjunctival {six (6) of six (6) and iridal (one (1) of six (6)}changes were seen in unrinsed rabbits primarily at the 1-hour inspection. All adverse findings were resolved by 72-hours except for one (1)rabbit with conjunctival redness which resolved by 7-days.The rinsed group exhibited some conjunctival irritation up to 72-hours. Irritation mean scores for unrinsed rabbits ranged from 8.2 (1-hour) to 0.33 (72-hours) to 0.0 (7-Days). Rinsed rabbits scores were 5.3 (1-hour) to 0.0 (72-hours). The test substance produced a mild irritation in rabbit eyes which was shown to be reversible. The test substance is considered to be a "MILD IRRITANT" to the eye.

Reliability: (1) valid without restriction

(25)

5.3 Sensitization

Type:	Guinea pig maximization test			
Species:	guinea pig			
Concentration:	Induction	5	% active substance	intracutaneous
	Induction	100	% active substance	intracutaneous
	Challenge	25	% active substance	occlusive epicutaneous
Number of Animals:	36			
Vehicle:				
Result:	sensitizing			
Classification:	sensitizing			
Method:	OECD Guide-line 406 "Skin Sensitization"			
Year:	1995		GLP: yes	
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)			

Method: Two (2) range finding trials (topical and intradermal injection) in two (2) male and two (2) female shaved albino guinea pigs were run which showed that the test substance at concentrations of 100% and 5% were appropriate for the definitive study, respectively. In the induction phase of the test, twenty test animals were given pairs of intradermal (0.1 mL) injections of 1) Freund's adjuvant, 2) %5 test substance in 0.5% acetone in propylene glycol, and 3) test substance + Freund's adjuvant at opposite sites from the animals' dorsal midline on Day-0. Appropriate negative and positive {2,4-Dinitro-1-chlorobenzene(DNCB)}controls were run on other animals. Topical induction exposures (48-hours) with site occlusion were done 7-days later following 24-hours test site exposure to Sodium lauryl sulfate.

Challenge (dermal)exposures were performed on Day-21 with both 25% (in acetone/mineral oil) and 100% test substance for 24-hours. Test animals were graded for dermal signs on the first and 2nd days following the challenge dosing. A dermal rechallenge trial was conducted on Day-28 by applying the test substance(25 and 100%) to these same animals. Dermal examinations were again performed one (1) and two (2) days later.

Result: The test substance induced no adverse clinical signs. Weak skin responses (erythema and edema) were observed in 25% test substance-treated challenge controls and in test substance-induced animals. Mean scores were not significantly different from the controls although a greater number of induced animals exhibited "slight but confluent or moderate patchy erythema". The test substance at 100% produced the same results. However, upon rechallenge of these animals 7-days later with 25 and 100% test substance, severities of dermal responses increased in test substance induced animals as did the mean dermal scores (0.8-1.0) relative to challenge (non-induced) controls (0.0-0.3). The positive control agent (DNCB) produced dermal scores at 24- and 48-hours of 0.3 and 0.5 for previously untreated animals versus scores of 2,5 for DNCB-induced guinea pigs. The test substance is considered to be a contact sensitizer.

Reliability: (1) valid without restriction

(24)

5.4 Repeated Dose Toxicity

Species:	rat	Sex: male/female
Strain:	Fischer 344	
Route of admin.:	oral feed	
Exposure period:	28 days	
Frequency of treatment:	Daily	
Post. obs. period:	2 weeks	
Doses:	0, 7.5, 30 and 120 mg/kg/day	
Control Group:	yes, concurrent vehicle	
NOAEL:	7.5 mg/kg	
LOAEL:	30 mg/kg	
Method:	other: Oral 4-week dietary study	
Year:	1996	GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)	

Method: The test substance was prepared by grinding in a coffee mill, sieved through a 125 um mesh screen and mixed with rodent diet NIH-07 at 0, 120, 470, 1900 ppm (0, 7.5, 30, and 120 mg/kg/day). Stability, homogeneity, and dose verification were performed to confirm compliance with protocol. The prepared dosed feed was presented to 14 male and 14 female rats (Fischer 344) per test group at twelve weeks of age for four (4) weeks. Six (6) rats/sex/group were held for post-exposure in two (2) week recovery groups. Test rats were monitored for body weights, feed consumption, and clinical signs. Collections were performed on six (6) or three (3) rats/sex/group at 28-days and 42-days sacrifice periods for blood (hematologies and clinical chemistries) and urinalyses, respectively. Necropsies were performed on all rats, and organs were weighed (liver, kidneys, pituitary, uteri, heart, brain, spleen, thyroids, adrenals, testes, and ovaries). These and other major organs were preserved in formalin, stained with H&E, and subjected to microscopic evaluations. Liver, kidney, and urinary bladder slices were subjected to immunohistochemical staining for proliferating cell nuclear antigen (PCNA) for assessment of cellular division.

Result: The test substance was shown to be completely stable in diets for 46-days. Mixing procedures produced homogeneous diets that were found within 10% of target concentrations. No compound-related deaths occurred. The body weights were not affected in male rats whereas the high dose female rats displayed 5% body weight decreases during study weeks two (2) through four (4). Food consumption was decreased in the high dose males and in the mid- and high dose females mainly during study weeks two (2) through four (4).

Various test substance-induced hematological changes occurred that included: increased mean corpuscular volumes and decreased mean corpuscular hemoglobin concentrations (high dose males and females) and blood bilirubin and cholesterol increases (high dose males and females). Most blood endpoints tended to approach control levels during week two (2) of the recovery period. No dose-related urinary changes were seen. Organ weight increases were seen at 28-days for liver and kidneys(high dose males and females; mid-dose females) and heart and spleen (high dose females). Only the kidney weights did not reach control levels by 42-days. There were no gross tissue or microscopic changes related to the test substance. Proliferating cell nuclear antigen (PCNA) exams showed cell division changes for: increases for liver cells (High dose males and females and mid-dose males at 28-days only); changes for kidney cells (decreases in high dose females at 28-days and increases in high dose males and females at 42-days; and increasing trend in urothelial cells in bladder (low and mid-dose males and females at 28-days). Macrocytic anemia was the primary change in rats related to the test substance administration. This change was reversible within 2 weeks following dietary exposure as were liver weight and serum cholesterol elevations. These changes were very minor, and had no apparent toxicological significance in this study. The lack of dose-responsiveness in the PCNA data provides results of uncertain importance to the assessment of the toxicity of this test substance.

Reliability: (1) valid without restriction
02-AUG-2000

(11)

Species:	rat Sex: male/female
Strain:	other: Fischer 344/N TacfBR
Route of admin.:	gavage
Exposure period:	21 days
Frequency of treatment:	Daily
Post. obs. period:	
Doses:	0, 0.1, 0.3, 1.0, and 3.0 g/kg/bw
Control Group:	yes, concurrent vehicle
LOAEL:	100 mg/kg bw
Method:	other: Oral 3-Week Range-Finding Study
Year:	1994 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Remark: A 4-week diet-study was also conducted.

Result: Doses of 1.0 and 3.0 g/kg/day of WINGSTAY 100 (mixed diaryl-p-phenylenediamines) were administered by gavage for up to 6 days were lethal for male and female F344 rats. The only pertinent gross finding of all unscheduled deaths was the paleness of most external surfaces and viscera. The mid-low (0.3 g/kg/day) and low(0.1 g/kg/day) doses caused time and dose related significant body weight loss, liver weight increase and hepatocellular labeling index increase at 0.1 g/kg. Therefore, in the subchronic studies, the recommended daily dose of WINGSTAY 100 (mixed diaryl-p-phenylenediamines) should not exceed 100 mg/kg/day, if administered by gavage.

Test substance Preparation: The test substance was prepared in an olive oil suspension for dosing.

Reliability: (1) valid without restriction
02-AUG-2000

(5)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test

System of testing: Ames/E. coli preincubation; Salmonella typhimurium TA-98, 100, 1535, 1537, 1538, and WP2 uvrA

Concentration: Salmonella stains without S9 activation: 0.167, 0.5, 1.67, 5, 16.7, and 50 ug/plate; Salmonella strains with S9 activation: 1.67, 5, 16.7, 50, 167, and 500 ug/plate; E.coli with/without S9 activation: 1.67, 5, 16.7, 50, 167, and 500 ug/plate

Metabolic activation: With and without

Method: other: Japan's Industrial Safety & Health Law, a combination of OECD Guidelines 471 and 472.

Result: Positive. The test substance was shown to cause mutations in Ames/Salmonella strains TA1538 and TA98 with S9 activation.

In a preliminary assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA1537, TA98, TA100, and WP2 uvrA with S9 metabolic activation, and in tester strains TA1535, TA1538, TA98, TA100 and WP2 uvrA without S9 activation, approximated the concurrent negative controls. However, statistically significant, increases in reverent frequencies, to approximately 1.7- to 2.5-fold control values, were observed in tester strain TA1538 with S9 metabolic activation and in tester strain TA1537 without S9 metabolic activation. In addition, the increases observed in strain TA1538 with S9 metabolic activation were dose dependent.

In a confirmatory assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA100, and WP2 uvrA with metabolic activation, and in tester strains TA1535, TA1538, TA98, TA100, and WP2 uvrA without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1537, TA1538, and TA98 with metabolic activation. Statistically significant increases in reverent frequencies, to control values, also were observed in tester strain TA98 without S9 metabolic activation. However, these latter increases apparently were not dose related.

The test substance was re-evaluated in all five Salmonella strains with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation. Reverent frequencies for all doses of the test substance in tester strains TA1535, TA1537, and TA100 with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation, approximated or were less than control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1538 and TA98 with S9 metabolic activation. All positive and negative control values in all assays were within acceptable limits.

Year: 1993 GLP: yes

Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
04-AUG-2000

(16)

Type: Ames test

System of testing: Ames/Salmonella-E.coli Liquid Pre-incubation Assay in Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 And in E.coli strain WP2 uvrA.

Concentration: Salmonella strains with S9: 1.67, 5, 16.7, 50, 167, and 500 ug/plate; Salmonella strains without S9: 0.167, 0.5, 1.67, 5, 16.7, and 50 ug/plate; E.coli with/without S9: 1.67, 5, 16.7, 50, 167, and 500 ug/ plate.

Metabolic activation: With and without

Method: other: Japan's Industrial Safety & Health Law, a combination of OECD Guidelines 471 and 472.

Result: Positive. The test substance was shown to cause mutations in Ames/Salmonella strains TA1537, TA1538 and TA98 with S9 metabolic activation.

In a preliminary assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA1537, TA100, and WP2 uvrA with and without S9 metabolic activation approximated the concurrent negative controls. However, statistically significant, increases in reverent frequencies, to control values, were observed in tester strains TA1538 and TA98 with S9 metabolic activation. In addition, the increases observed in strain TA1538 with S9 metabolic activation were dose dependent.

In a confirmatory assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA100, and WP2 uvrA with metabolic activation, and in tester strains TA1535, TA1537, TA1538, TA100, and WP2 uvrA without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1537, TA1538, and TA98 with metabolic activation. Statistically significant increases in reverent frequencies, to control values, also were observed in tester strain TA98 without S9 metabolic activation. However, these latter increases apparently were not dose related.

The test substance was re-evaluated in all five Salmonella strains with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation. Reverent frequencies for all doses of the test substance in tester strains TA1535, and TA100 with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1537, TA1538, and TA98 with S9 metabolic activation. All positive and negative control values in all assays were within acceptable limits.

Year: 1994 GLP: yes

Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
04-AUG-2000

(17)

Date: 22-Jan-2003
5. Toxicity ID: 68953-84-4

Type:	Cytogenetic assay
System of testing:	Chromosomal aberration assay in CHO cells
Concentration:	0.4, 2, 4, and 25 ug/mL
Metabolic activation:	With and without
Result:	Negative. The test substance was judged negative (non-clastogenic)based on its inability to reproducibly induce dose-related increases in structural chromosomal aberrations in CHO cells. Analysis of the data for the 24-hour treatment with the test substance indicated that there were statistically significant dose-related increases in the frequency of aberrations/cell and proportion of aberrant metaphases at doses 2 and 4 ug/mL. The data for the 2 and 4 ug/mL doses produced a statistically significant linear trend when analyzed by the Cochran/Armitage Linear Trend Test. To verify the biological significance of this finding, the 24-hour treatment was repeated. In the confirmatory assay, the test substance was re-evaluated at doses of 25 ug/mL with S9 metabolic activation (5-hour treatment) and 0.4, 2, and 4 ug/mL without S9 metabolic activation (24-hour treatment). Analysis of the data for the 5-hour treatment did not produce statistically significant increases in aberrations/cell or in proportion of aberrant metaphases. Analysis of the data for the 24-hour treatment indicated a statistically significant increase in aberrations/metaphase at the mid-dose (2 ug/mL) with S9 metabolic activation but there were no significant increases in the proportion of aberrant metaphases. However, when the data for 2 ug/mL (0.045 + or - 0.208) were compared to the untreated control data (0.025 + or - 0.157) or to Pharmakon historical acetone data (0.034 + or - 0.021), there were no statistically significant increases in the frequency of aberrations/metaphase. Therefore, the positive finding in the t-test for 2 ug/mL was considered a statistical artifact with no biological significance. There were no other statistically significant increases in aberration/metaphase or in the proportion of aberrant metaphases at any of the remaining dose levels for the 24-hour treatment.
Method:	OECD Guide-line 473 "Genetic Toxicology: In vitro Mammalian Cytogenetic Test"

In the structural Chromosomal Aberration assay, duplicate cultures were established for each dose level. Three treatment schedules were used: a) First set of cultures were treated for 5-hours with the appropriate dose of the test sample in Ham's F12 serum free (F12SF) medium either in the presence or absence of S9 metabolic activation along with concurrent negative and positive controls followed by three (3) Puck's saline washes and medium replacement; b) Second set of cultures were treated for 24-hours with the test substance or control articles in Ham's F12 medium containing five (5) % serum (F12FCM5%) without S9 metabolic activation, and; c) Third set of cultures were treated for 48-hours with the test substance or control articles in F12FCM5% medium without S9 metabolic activation. Two (2) to three (3) hours prior to harvest, Colcemid (2X10-7M) was added to all sets of cell cultures to arrest dividing cells in metaphase. CHO cells were harvested at the appropriate time and metaphase slides were prepared and stained.

The data from one hundred metaphases from each culture (200 metaphases per dose point) were pooled for statistical analysis. Data were evaluated by using the chi-square of aberrant versus normal cells while comparing each dose level to its concurrent negative control. The data were also analyzed for statistical significance by pairwise t-tests comparing the number of aberrations per cell in each treated dose versus the negative control.

Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
20-FEB-2001

(19)

Type: DNA damage and repair assay
System of testing: E. coli Pol A1- Liquid Suspension Assay

Concentration:

Metabolic activation: Without

Result: Positive
Method: Other
Year: 1980 GLP: no

Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000

(33)

Type: other: Transformation Assay
System of testing: Balb/3T3 In Vitro Transformation Assay

Concentration: .01 ug/ml to 1.0 ug/ml

Metabolic activation: Without

Result: Negative
Method: other
Year: 1981 GLP: no

Test substance: Nailax (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.
04-AUG-2000

(12)

Type: other: Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes
System of testing: Hepatocytes form male Fischer 344 (F344/Crl) rats

Concentration: Slightly above their limits of solubility

Metabolic activation: Without

Result: Negative. In all the Unscheduled DNA Synthesis Assay (UDS) trials, the three (3) negative controls {the untreated cells control, F, and Dimethylsulfoxide (DMSO)} had negative values for Net Nuclear Gain (NNG) counts (<0). A positive control, 2-Aminofluorene (2-AF) was positive for induction of UDS; the mean NNG counts were 45.92 and 58.99 in the first and second assays, respectively, indicating assay validity. (i.e., hepatocytes were capable of metabolic activation and DNA repair). The positive control responses occurred at

toxic levels. UDS assay results for NNGs were in the range of -26 to -46, demonstrating a lack of UDS activity for the three (3) condensation products at concentrations greater than their solubilities in the test media. The results indicated that, under controlled laboratory conditions, the condensation products from the reaction of 1.4-Benzenediamine, N,N', mixed Ph and tolyl. derivs. with Dicyclopentadiene were negative for induction of UDS in rat hepatocytes at concentrations up to and greater than their solubilities. This assay demonstrated a lack of genetic activity in this mammalian DNA-repair test system.

Method: other: Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes on Test substance Condensation Products. The test substance, 1,4-Benzenediamine, N.N'-mixed Ph and tolyl. derivs., was reacted with Dicyclopentadiene in varying ratios, resulting in three condensation products. Each of these condensation products were subjected to independent in vitro unscheduled DNA synthesis (UDS) assays with hepatocytes from male Fischer 344 (F344/Crl) rats. All three (3) condensation products were tested at concentrations slightly above their limits of solubility in the tissue culture medium. Hepatocytes were exposed to test substances for 18-20 hours to allow bioactivation and DNA repair. The assay was based on the incorporation of 3H-thymidine into the hepatocyte's DNA during repair of DNA-damage. This incorporation was monitored by counting Net Nuclear Grains (NNG) formed on photographic emulsion placed on the cells adhering to glass slides. Criteria for a positive response included : (a) Significant increase in number of grains at two (2) levels of exposure above negative control levels, (b) A dose-responsiveness in grain counts up to toxic levels of exposure, and (c) At least one (1) value for NNG that is five (5) or above. A negative response is reported for NNG's that are <0, and an equivocal or inconclusive response are results that are 0<#<5.

Year: 1999 GLP: yes

Test substance: The test substance, 1,4-Benzenediamine, N.N'-mixed Ph and tolyl. Derivs. condensation products with Dicylopentadiene

Reliability: (1) valid without restriction
07-AUG-2000

(37)

5.6 Genetic Toxicity 'in Vivo'

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex:
Strain:
Route of admin.: Oral feed
Exposure period: 24 hours
Doses: 50 ug/ml and 10 ug/ml
Result: Negative. Negative under conditions of the assay
Method: other: Drosophila melanogaster (Fruit Fly) System
Year: 1979 GLP: no

Test substance: Nailax B (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000

(32)

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex:
Strain:
Route of admin.: Oral feed
Exposure period: 24 hours
Doses: 0.05 mg/ml and 0.63 mg/ml
Result: Negative. Negative under conditions of the assay
Method: other: Drosophilia SLRL Assay
Year: 1979 GLP: no

Test substance: Nailax (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000

(13)

Date: 22-Jan-2003
5. Toxicity ID: 68953-84-4

Type: Micronucleus assay
Species: Mouse Sex: male/female
Strain: CD-1
Route of admin.: i.p.
Exposure period: single dosing
Doses: 0, 250, 1250, 2500 mg/kg test chemical; 0.5 g/kg TEM (+ control)
Result: Negative. There were no statistically significant depressions in the PCE/NCE ratios in any groups of mice except for the 2500 mg/kg group at 48-hours sacrifice time ($p<0.01$) which was an indication that the test substance had reached the bone marrow and was toxic to erythrocytes.

Analysis of the micronucleus data for the groups treated with the test substance indicated that there were no statistically significant increases in the frequency of micronucleated PCEs. The test substance was judged negative (non-clastogenic) based on its inability to induce micronucleated PCEs.

Method: OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"

Nine (9) groups of mice (CD-1) were acclimated to laboratory conditions for 25-days prior to initiation of the study. The mice were randomized by body weight and assigned to groups using a computer-generated random number list.

Each group of mice was comprised of ten (10) animals(five (5) males/five (5) females). Each mouse received a single interperitoneal dose at 10 mL/kg of body weight. The test substance at dose levels of 250, 1250, and 2500 mg/kg was administered to three (3) groups of mice which were sacrificed at 24-, 48-, and 72-hours post dose. Concurrently, the negative control, Dimethylsulfoxide (DMSO)/corn oil, was administered, as dose volume of 10 mL/kg of body weight, to three (3) groups of mice. A group of these mice were included in each sampling time. The positive control, Triethylenemelamine at 0.5 mg/kg, was administered to one (1) group of mice and sacrificed at 24-hours post dose.

All mice were sacrificed and their femurs were removed. Their bone marrow was removed by flushing. Smears were made of the suspended cells.

One (1) thousand young erythrocytes were evaluated for a change of ratio of polychromatic erythrocytes (PCE) to normochromatic cells (NCE).

Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
04-AUG-2000

(18)

Type:	Other: 32P Postlabeling Assay for Detection of Adduct Formation in Rat DNA
Species:	rat Sex: male/female
Strain:	other: Fischer 344/N TacfBR
Route of admin.:	Gavage
Exposure period:	7 days
Doses:	0., 0.3, 1.0, and 3.0 g/kg/bw
Result:	Negative. Under conditions of the study, the test substance did not induce DNA-adducts in the liver and urinary bladder DNA of rats.
Method:	Other: 32P Post-Labeling Assay for DNA Adduct Formation
Year:	1995 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)
Remark:	The purpose of the study was to determine the potential of WINGSTAY 100 (mixed diaryl-p-phenylenediamines)to bind covalently to liver and urinary bladder DNA of male and female rats after in vivo administration of WINGSTAY 100.
Result:	Under conditions of the study, the test substance did not induce DNA-adducts in the liver and urinary bladder DNA of rats.
Reliability:	(1) valid without restriction
07-AUG-2000	

(4)

5.7 Carcinogenicity

Species:	rat	Sex: male
Strain:	Fischer 344	
Route of admin.:	oral feed	
Exposure period:	38 weeks	
Frequency of treatment:	Daily	
Post. obs. period:		
Doses:	1900 ppm	
Result:	Negative. The test substance exerted toxicity to the erythropoietic system, but there was an absence of tumor initiating or promoting activity.	
Control Group:	yes, concurrent vehicle	
Method:	other: Accelerated bioassay (ABA)	

The accelerated bioassay (ABA) was conducted on male F344 rats for 38 weeks. The target sites chosen for the ABA were liver and urinary bladder and the dose of the test substance was 1900 ppm as previously established to be a toxic dose. The liver tumor initiator was Diethylnitrosamine (DEN) and the urinary bladder initiator was N-Butyl N(4-hydroxybutyl)nitrosamine(BBN). The initiators, which included the test substance as a possible initiator, were administered during the first 14-weeks followed by the promoters. The promoters, Phenolbarbital (PB) for the liver and Nitrilotriacetate (NTA)for the urinary bladder and the test substance as a possible promoter, were administered during last 24-weeks after the test substance. The study had 11 test groups, including a negative control. The critical comparisons for initiation activity were conducted between Group Three (3) (PB) and Group Six (6) (Test substance + PB) for the liver and Group Eight(8) (NTA) and Eleven (11) (Test substance + NTA) for the urinary bladder. The critical comparisons for promoting activities were conducted between Group Two (2)(DEN) and Group Five (5) (DEN + Test substance) for the liver and Group Seven(7) (BBN) and Group Ten (10) (BBN + Test substance) for the urinary bladder. There were 26- and 38-week sacrifices.

Once daily, clinical observations were made and on scheduled body weighing days, a thorough palpation was performed on all animals. Body weights were recorded weekly from the first week of dosing until scheduled sacrifice at 26-weeks, and every 2-weeks thereafter.

At the two (2) scheduled sacrifices, all animals were subjected to a complete gross postmortem examination, The liver and kidneys were weighed. Liver, urinary bladder, kidneys and any grossly observed change or lesions were sampled, fixed, processed, cut and stained for microscopic examination. Tissue samples were taken from each of the three (3) liver lobes. NBF was used to inflate the urinary bladder at necropsy. All animals found dead or those killed in extremis were submitted to a complete gross postmortem examination. No organ weights were taken. The mean number of neoplasms per animal, the biggest diameter of carcinomas (in mm), the average diameter of carcinomas (in mm), and the degree of severity of carcinomas were recorded.

In order to assess proliferation, separate liver and urinary bladder sections were fixed in NBF, were cut and stained for PCNA. Subsequently, they all were aquatinted according to the method described above.

Statistical analyses were performed on weekly body weights, final body weights, absolute and relative liver and kidney weights, tumor incidence and PCNA data using methods described above.

Year: 1996 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines) The test substance was prepared in an olive oil suspension and mixed with rodent diet NIH-07 for dosing.

Reliability: (1) valid without restriction

(2)

Species: rat Sex: male/female
Strain: Fischer 344
Route of admin.: oral feed
Exposure period: 52 weeks
Frequency of treatment: Daily
Post. obs. period: 12 weeks
Doses: 53, 310, 1900 ppm
Result: Negative. No test substance related deaths occurred, although the high dose of 1900 ppm caused a decrease in body weight gain and food consumption in both genders. Red blood cell mean corpuscular volume was significantly increased at 38-weeks, accompanied by a significant decrease in mean corpuscular hemoglobin concentration.

At 52-weeks, the red blood cell count and hemoglobin values were also significantly decreased in high dose animals of both genders. Total bilirubin and cholesterol were increased in high dose animals at 38- and 52-week sacrifices. During the 3-month recovery, hematology parameters, bilirubin and cholesterol returned to control values. Total protein was reduced in high dose animals of both genders, throughout the entire exposure and recovery periods. The test substance also produced increases in relative liver, spleen, heart, and kidney weights in high dose animals. Both genders of all test substance groups exhibited significant increases in urothelial cell proliferation (measured by PCNA)and adaptive hyperplasia. No regenerative hyperplasia, prenoplasia, or neoplasia were present. There were microscopic evidence of extramedullary erythropoiesis in the spleen and liver of high dose animals in both genders; otherwise, no other pertinent microscopic findings were evident. The test substance exerted toxicity to the erythropoietic system, but displayed no carcinogenic activity.

Control Group: yes, concurrent vehicle

Method: other: One year study in male and female F 344 rats

The study used both genders of Fischer 344 (F344/N Tacf Br MPF) rats. There was a 38-week interim sacrifice in addition to 52-week, and 12-week post-exposure (recovery) sacrifice periods. The high dose in the study (1900 ppm) was the maximum tolerated dose identified in subchronic studies, in which there was no observable gender difference.

Once daily, cage side clinical observations were made, and on days scheduled for body weighing, a thorough body palpation was performed. Body weights were recorded one (1) week prior to initiation of exposure, weekly for weeks 1-13, and once every two (2) weeks thereafter. Food consumption was measured for weeks 1-13, and once every two (2) weeks thereafter. Indirect ophthalmoscopy was performed on all animals prior to exposure and during week-52.

During the three (3) sacrifices (at 38-, 52-, and 64-weeks), Five (5) rats/group/gender were used for hematology, clinical chemistry, and urinalysis. At scheduled sacrifices, all animals were subjected to a complete postmortem examination. Key organs were weighed and the tissues fixed in neutral buffered formalin (NBF), processed, cut, and stained with H&E. Tissue samples were taken from each of the three (3) liverlobes. NBF was used to inflate the urinary bladder at necropsy. All animals found dead and those killed in extremis were submitted to a complete gross postmortem examination. For these, no organ weights were taken, but all grossly observed changes and all key tissues were examined microscopically.

To assess cell proliferation, separate liver, urinary bladder and kidney sections were fixed in NBF, cut, and stained for proliferating cell nuclear antigen (PCNA). The quantitation of PCNA-positive nuclei in the immuno-stained sections of these tissues, was performed from 38-, 52-, and 62-week sacrifices. Next, the proliferation index (PI) for the liver, urinary bladder, and kidney for each animal was calculated, representing the percentage of PCNA-positive nuclei out of the total number of hepatocellular, urothelial, or tubular nuclei counted. The results were subjected to appropriate statistical analysis.

Statistical analysis was performed on weeking body weights, food consumption data, absolute and relative organ weights, hematology, clinical chemistry, urinalysis, and PCNA data.

Year: 1996 GLP: yes

Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines) The test substance was prepared in an olive oil suspension and mixed with rodent diet NIH-07 for dosing.

Reliability: (1) valid without restriction

(3)

5.8 Toxicity to Reproduction

Type: Two generation study
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure Period: F0 exposed during 10 weeks premating, 2 weeks of mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 males and females exposed for 10 weeks prior to mating.
Frequency of treatment: Daily
Premating Exposure Period:
male: 10 weeks
female: 10 weeks
Duration of test: 9 months
Doses: 0, 120, 400 or 1500 ppm.

Control Group: yes, concurrent no treatment

Method: OECD Guide-line 416 "Two-generation Reproduction Toxicity Study"

This study was designed in compliance with EPA GLP and USEPA FIFRA guidelines. Dose levels were established from a Range finding study at Research Triangle Institute which employed dietary levels of 120, 1900, and 5700 ppm of WINGSTAY 100 (mixed diaryl-p-phenylenediamines). The top level was lethal to dams and offspring, 1900 ppm induced one nonviable litter in 9 total, and thus, the top dose for the definitive study was decreased by 20% to assure high viability in test group. No effects were seen at 120 ppm.

This study used 30 Sprague Dawley rats/sex/dose (F0) exposed to diets containing 0, 120, 400 or 1500 ppm WINGSTAY 100 during 10 weeks premating, 2 weeks mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 litters were culled to 10 each at 4 days postnatal (PND) 30 other F1 males and females/group chosen for pairing, and fed WINGSTAY 100 as above for 10 weeks prior to mating. After mating/gestation of F1, the resulting F2 rats were delivered, and maintained through weaning period (to PND 21). Weekly body weights (BWs) and food consumption (FC), and daily clinical observations were recorded. Necropsies and histopathology (primary kidneys) were performed on selected rats from each sex/group/generation (all F0 and F1 dams at PND21, three F1 and F2 pups/test group at PND21). Remaining F1 and F2 rats were euthanized without examination. Data were collected on vaginal cytology, mating, pregnancy, litter, and pup parameters.

Remark: WINGSTAY 100 induced dystocia (difficult deliveries) in pregnant rats which may have led to prolonged gestation and increased perinatal deaths, decreased live births, and increased pup weights. In addition, polycystic lesions were observed at all dose levels. Prolonged gestation has previously been associated with the WINGSTAY component DPPD, and polycystic kidneys were observed in DPamine-treated rats. Based upon adult toxicities, reproductive and offspring endpoints, there was no NOEL for WINGSTAY 100 in this study.

Result: High dose females had decreased Body Weights (BWs) relative to other test groups throughout majority of study period. Mortality during gestation/lacation were: F0 dams- 0 in 24 pregnancies, 0/27, 3/24, 4/25; F1- 0/22, 0/23, 1/22, 1/24. Numbers of pregnancies with no live births: F0- 0, 1, 1, 10; F1- 0, 1, 1, 2. Gestational length: F0- 22.2 days, 22.4 days, 22.8*, 23.5*; F1- 22.2, 22.8*, 23.1*, 23.2* (* = statistically significant). The number of live pups/litter: F0-15.6, 14.1, 11.9, 7.6*; F1- 15.6, 13.7, 13.3, 10.8*. Pups weights (g) on PND 0: F0- 6.38, 6.79*, 6.93*, 6.63*; F1- 6.32, 6.89*, 6.99*, 6.63*.

WINGSTAY 100-related kidney lesions were observed grossly (as white or clear cysts) and microscopically (polycyctic findings with variable severity): F0 adults-males 0/0, 0/0, 0/0, 0/1 and females 0/0, 0/0, 0/2, 3/9; F1 weanlings-males 0/23, 1/25, 8/20, 10/11 and females 0/22, 5/26, 7/18, 11/11; F1 adults-males 0/30, 5/30, 10/30, 21/30 and females 0/30, 2/30, 1/30, 18/30; F2 weanlings-males 0/60, 3/64, 6/19, 15/16 and females 0/60, 5/64, 8/19, 15/15. The severity of kidney lesions were also dose related.

Year: 2000 GLP: yes

Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines) The test substance was prepared in an olive oil suspension and mixed with rodent diet NIH-07 for dosing.

Reliability: (1) valid without restriction
11-FEB-2001

(36)

Type: Two generation study
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure Period: F0 exposed during 10 weeks premating, 2 weeks of mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 males and females exposed for 10 weeks prior to mating.
Frequency of treatment: Daily
Premating Exposure Period:
male: 10 weeks
female: 10 weeks
Duration of test: 9 months
Doses: 0, 120, 400 or 1500 ppm.

Control Group: yes, concurrent no treatment

Method: Other: Derivation of Benchmark Dose from 2-Generation Rat Study
Test Substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Bench Mark Responses (BMR) are estimations of doses inducing a discrete toxic response in a test population at an incidence within the range of 1-10%. The Bench Mark Dose (BMD) is represented as the 95% lower confidence limit (LCL) for a BMR, or as a Most Likely Estimate (MLE). In this project, data from the 2-generation reproduction study in rats on Wingstay 100 (RTI #65C-6429-400)(36) chosen for analyses were the (1) polycystic kidney lesions in F1 male adults and F1 female weanlings, and (2) gestational lengths (days) for F1 pregnant females.

Data for these endpoints at the 3 dose levels employed in the study were subjected to various analyses including Gamma, Multistage, Quantal Linear, Weibull, Probit, Logistic, and Quantal Quadratic (for quantal data – polycystic kidneys), and Power, Linear, and Polynomial models (continuous data – gestational lengths). Estimations were also made to derive "best fit" information for each model run. The methodology employed was according to the "Benchmark Dose Technical Guidance Document" (1996), EPA/600/P-96/002A.

Results: Most Likely Estimate (MLE) and 95% Lowest Confidence Limit (LCL) values were derived for the most sensitive toxic endpoints (observed graphically). The models that "best fit" polycystic data for F1 male adults and F1 female weanlings were the quantal linear and multistage procedures. The BMD 10% values (EPA default for quantal data) derived for F1 male adults are 7 mg/kg-day (LCL) and 9.3 (MLE), and for F1 female weanlings, the values are 3.7 and 6.0 mg/kg-day, respectively. The prolongation of parturition analysis for F1 females indicated that none of the models produced a good fit although there was good agreement amongst the 3 models tested, giving BMD 5% estimations of 160 (LEL) and 226 (MLE) mg/kg-day for this endpoint.

The Bench Mark Dose (10% incidence) developed for the the most sensitive endpoint (polycystic kidneys in F1 female weanling rats) in the 2-generation rat dietary study was 3.7 (95% Lower Confidence Limit) and 6.0 (Most Likely Estimate) mg/kg-day. These numbers are below the lowest exposure levels (and LOEL) found in the 2-generation study, and thus pose plausible estimates of a 10% incidence rate for this endpoint. These calculations provide a credible low dose benchmark that can be used as a basis for safety assessments in exposed populations.

(40)

5.9 Developmental Toxicity/Teratogenicity

Species:	rat	Sex: female
Strain:	Sprague-Dawley	
Route of admin.:	gavage	
Exposure period:	10 days	
Frequency of treatment:	Dosed on days 6-15 gestation	
Duration of test:		
Doses:	0, 20, 70, 200 mg test material in 5 ml corn oil/kg	
Control Group:	yes, concurrent vehicle	
NOAEL Maternalt.:	70 mg/kg bw	
NOAEL Teratogen.:	<= 200 mg/kg bw	
NOAEL Fetal:	70 mg/kg bw	
Method:	OECD Guide-line 414 "Teratogenicity"	

Preliminary trials in 8 rats/group indicated that 600 mg/kg was lethal to 50% of maternal rats while 200 mg.kg caused decreased body weights in maternal and fetal animals. There were no effects at 20 or 70 mg/kg. Consequently, 200 mg/kg was selected as the top (high) dose in the definitive study, Confirmation of the test dose solutions were confirmed analytically.

The definitive study used 25 inseminated female rats per test group (0, 20, 70, and 200 mg of test substance/kg doses in five (5) mL corn oil/kg). The animals were dosed on Days 6-15 gestation. Body weights, food consumption, liver weights, clinical changes, pregnancy rates, and corpora lutea counts were followed along with numerous fetal parameters. All fetuses were weighed, sexed, and assessed for external and visceral abnormalities. One (1) half of the fetuses were examined for skeletal abnormalities while the second half were subjected to cranial bone assessments.

Remark: Administered in 5 ml corn oil/kg by gavage

Result: The test substance induced no lethality. Deficits were seen in maternal body weights (Day-12 and body weight change from Day-6 to Day-15) and food consumption (during treatment period) at the highest dose only (200 mg/kg). Pregnancy rates, litter sizes, number of live fetuses, uterine implantation, and all gestational parameters were unaffected by chemical treatment. There was a linear trend towards lower body weights in fetuses with increasing doses (approximately 5% decrease in 200 mg/kg group). Assessment of cranial, skeletal, visceral, and external appearance discerned no compound-related abnormalities (malformatiuons or variations) according to established criteria. The test material produced minimal effects (body weight) to maternal rats from oral dosing of 200 mg/kg during pregnancy. There was no induction by the test chemical of birth defects (major or minor) in fetal animals.

22-Jan-2003
5. Toxicity ID: 68953-84-4

Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (1) valid without restriction
08-AUG-2000

(22)

Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure period: Varied, see method
Frequency of treatment: Varied, see method
Duration of test:
Doses: 2500 ppm
Control Group: yes, concurrent vehicle
Method: other: Mechanistic Study

The toxicity of the test substance to maternal and 1st generation offspring was evaluated by exposing CD (Sprague-Dawley) rats to fixed dietary concentrations of 2500 ppm during different time periods (i.e. exposures during prebreed, mating, gestation, and/or lactation). Five (5) Groups (20/sex/Group) were studied including: Group one (1)- Negative control; Group two (2)- Dietary test substance during prebreed and mating, exposures ended on gestation day (gd)-0; Group three (3)- Dietary test substance during gestation and lactation, exposures began on gd-0; Group four (4)- Dietary test substance during prebreed, mating, gestation, and lactation, the Positive control and; Group five (5)- Dietary test substance during prebreed, mating, gestation, and lactation, plus 600 ppm of iron gluconate in the drinking water for prebreed through lactation.

Males and females were paired within Groups (1:1) for the two-week mating period. Once a given female was found to be sperm positive {date designated as gestation day (gd)-0}, "her" male was euthanized and discarded. On the day of delivery (pnd-0), pups were counted, sexed, and weighed. On pnd-4, litters were culled to ten, counted, sexed, and weighed. On pnd-7, -14, and -21, pups were counted, sexed, and weighed. All pups were euthanized and one (1)/sex/litter necropsied on pnd-21. Dead pups on pnd-0 and -1 were examined macroscopically (necropsied) for polycystic kidneys. Female body weights and feed consumption were recorded weekly during prebreed, gestation, and postnatally. At necropsy on pnd-21, the maternal spleen, liver, and kidneys were weighed and retained in a fixative. Kidneys form Groups one (1) and five (5) were examined histopathologically.

22-Jan-2003
5. Toxicity ID: 68953-84-4

Blood sampling was performed ongestation day-21 and pnd-21 from all females (pregnant) by tail vein withdrawal. Blood sampling was performed on pnd-21 on the F1 offspring by withdrawal from the abdominal vena cava at sacrifice. The blood parameters assessed were: WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, Platelets, WBC Differential (to correct the RBC and WBC counts for Nucleated Red Blood Cells) and Methemoglobin. On gd-21, a second sample of blood was taken via tail vein from all pregnant females in all Groups, with plasma frozen for possible subsequent analysis for specific hormones. For Group three(3), any female who had not yet delivered by gestation day-23 had blood taken from the tail vein and plasma frozen. On pnd-21, the spleen, liver, kidneys, and heart from one(1) pup/sex/litter were weighed and retained in a fixative.The kidneys from all offspring were examined histologically. Statistical analysis included both parametric and nonparametric tests for continuous and discrete data.

Remark: The objectives of this study were to confirm and further characterize previously-observed effects following the test substance administration to pregnant rats. This study was designed (1) to determine the necessary and sufficient timing of exposure to maternal females at a fixed dietary concentration of the test substance to produce dystocia, prolonged gestation, and polycystic kidneys in offspring, (2) to determine whether the test substance results in demonstratable macrocytic anemia in maternal animals, (3) to determine if there is treatment-induced anemia and whether iron supplementation ameliorates or prevents the anemia, dystocia, and/or polycystic kidneys, and (4) to determine if F0 parental females exhibit polycystic kidneys due to dietary exposure to the test substance.

Result: F0 Males: The test substance intake over the prebreed period (Study Days 0-28) averaged 180 mg/kg/day for all three (3) exposed Groups {two (2), four (4), and five (5)}. Iron gluconate intake in Group five (5) averaged 56 mg/kg/day (Study Days-0 to 28). Clinical observations were found to be unrelated to compound administration.

F0 Females: The test substance intake averaged 187-192 mg/kg/day for Groups two (2), four (4) and five (5) during gestation days (gd)-0 to 28. Iron gluconate intake during gestational days-0 to 28 in Group five (5) averaged 53 mg/kg/day. Clinical observations during gestation included one (1) female found dead in Groups three (3) and four (4), alopecia predominantly in Groups four (4) and five (5), pale eyes and tail, pale (not otherwise specified) almost exclusively in Groups three(3), four (4) and five (5) (all exposed), pilorection in Groups three (3), four (4) and five (5), and delayed parturition in Groups three (3), four (4), and five (5).

The hematological profile of maternal rats on gestation day-21 found no evidence on macrocytic anemia in any groups.

REPRODUCTIVE/DEVELOPMENTAL: Gestational index (a measure of live litters relative to pregnant females) was significantly increased in Groups three (3) and four (4) but not in Group five (5). Male mating, fertility, and pregnancy indices were equivalent across all groups. Gestational length in days was significantly prolonged in Group three (3) (23.6+/-0.2), Group four (4) (23.8+/-0.2), and Group five (5) (23.5+/-0.2) relative to Control Group value (22.2+/-0.1) and the value in Group two (2) (22.3+/-0.1). Number of implantation sites per litter was significantly reduced in Group five (5). Percent of post implantation loss was significantly increased in Groups three (3) and four (4). Pups per litter were significantly reduced in Groups three (3), four (4) and five (5), and number of dead pups per litter were significantly increased in Groups three (3) and four (4). Weanling gross and microscopic findings were limited to hydronephrosis in Groups one (1) and two (2), gas in intestines in Group two (2), and gross evidence of polycystic kidneys in Groups three (3), four (4), and five (5). Maternal hematologic profiles at sacrifice (21 days after delivery) indicated statistically significant changes in most erythrocyte parameters. The white blood cell differential counts indicated changes (as percent of cells examined) as follows: increase in segmented neutrophils and decrease in lymphocytes only in Group four (4), with no treatment-related changes in the percentages of monocytes or eosinophils. Histopathologic assessment was performed on kidneys of all maternal rats in Groups one (1) and five (5). Polycystic kidneys were observed microscopically (but not macroscopically) in three (3) of 20 animals in Group five (5), with no polycystic kidneys observed in Group one (1).

The timing of exposure to the test substance with respect to pregnancy is an important determinant of toxicity. Exposure of F0 females to 2500 ppm of the test material during gestation is necessary and sufficient to produce dystocia (prolonged gestation).

It is necessary and sufficient to expose F0 dams during gestation and/or lactation to produce polycystic kidneys in the F1 offspring. Since no Groups were exposed only during gestation or only during lactation, it is not possible to further define how exposure timing affects this endpoint. There was no demonstrable macrocytic anemia in gestation day-21 (gd-21) F0 dams in any treatment Group, but at post delivery day-21 (pnd-21), F0 mothers exposed prior to and during mating, gestation, and lactation were anemic. The F1 offspring at pnd-21 did not consistently display evidence of macrocytic anemia. Iron supplementation did not affect pnd-21 maternal anemia, dystocia, or incidence/severity of polycystic kidneys in the F1 offspring. However, perinatal survival of the offspring was affected. Microscopic, but not macroscopic evidence of polycystic kidneys was found in 15 percent of dams treated prior to and during mating, gestation, and lactation (with iron supplementation). Controls had neither macroscopic nor microscopic indications of polycystic kidneys. Exposure of animals to the test substance prior to and during mating {Group two (2)} did not appear to result in adverse affects to offspring. Furthermore, exposure during the prebreed/mating periods did not increase the affects produced from gestation/lactation exposures only.

Year: 2000 GLP: no
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although this study was not conducted to GLP, the test parameters used were based on a sound scientific design.
09-AUG-2000

(15)

5.10 Other Relevant Information

Type: other: A Photoirritation Study in Rabbits
Method: US FDA test guidelines and GLPs.
Result: UV light did not enhance the skin irritation response of the test substance in rabbits, and therefore is not considered to be a photo-irritant.

Test condition: Albino rabbits (4 females,4 males) were shaved in the dorsal portion of the animals trunk. One day later, 0.5 g of test material was placed onto 2 skin site of 3 male and 3 female rabbits. 0.5 ml of Oxsoralen lotion was similarly applied to 1 male and 1 female rabbit. After 2-hour skin contact exposure period, the gauze patches were removed from the animals' right sides and the left side sites were covered with aluminum foil to prevent light exposure. All animals were exposed to UVA light for 40 minutes. Following light exposures, the gauze patches were reattached for additional 21 hours.

Skin sites were scored according to Draize procedures at 25, 48 and 72 hours plus 7 days following cessation of chemical exposure.

Reliability: (1) valid without restriction

(1)

other: Mechanistic

Method: Dietary WINGSTAY 100 (mixed diaryl-p-phenylenediamines) induced dystocia and delayed parturition with associated maternal deaths in pregnant rats in a 2-generation reproduction study. This mechanistic study was designed to assess exposure conditions necessary to induce these findings, and the role of possible iron deficiency. Female rats were exposed to 2500 ppm of WINGSTAY 100 in the diet as follows:

Group 1- 0 ppm for 12 week study (negative control)
Group 2- Exposed 4 weeks prebreed plus 2 weeks mating
Group 3- Exposed 3 weeks gestation plus 3 weeks lactation
Group 4- Exposed 4 weeks prebreed, 2 weeks mating, 3 weeks gestation, 3 weeks lactation (positive control)
Group 5- Positive control plus iron supplementation (600 ppm iron gluconate in drinking water)

Females (20/group) were mated with males with comparable dietary exposures. Following confirmed mating, males were sacrificed without further assessment. Rats were subjected to daily observations, weekly Body Weights (BWs), and feed and water consumptions. Maternal F0 rats were bled on gestational day 21 prior to delivery and post delivery day 21. A sample of plasma was frozen from the gestation day 21 bleeding for possible future endrocrine assessments. F1 rats were bled on day 21 post natal. Samples were subjected to standard hematology and metHgb assays. Major organ weights were determined. Observations were made during reproductive, gestational, and postnatal periods of the study. Necropsies with organ weights determinations were performed on all surviving F0 and F1 rats 21 days post delivery. Microscopic exams were performed on gross lesions in F0 rats, and on kidneys of F0 and F1 animals.

Remark: The study confirmed results in a 2-generation reproduction rat study that demonstrated dietary WINGSTAY 100 induces dystocia, delayed parturitition, and an associated decrease in pup survival at birth.

These findings have earlier been associated with DPPD and DPA according to available literature. The effects in Group 3, but not Group 2 indicate that chemical exposure during gestational period is essential
for the dystocia and delayed parturition observed. Since Group 3 included exposure during lactation, it is uncertain whether gestational exposure alone would induce the polycyctic kidneys in offspring. Pre-gestational exposure did not enhance the effects attributed to gestational WINGSTAY 100 ingestion. Finally, although iron supplementation had no apparent impact on blood parameters, it did decrease the number of stillbirths without impacting other reproductive or litter endpoints.

Result: Body weights and feed consumption for F0 rats were reduced relative to negative controls, possibly as a result of decreased palatability of the WINGSTAY 100-containing diet. One (1) Group 3 female died on gestation day 19, and one (1) Group 4 rat on gestation day 24. Due to dead litters, additional Groups 3 and 4 dams were euthanized. Other clinical observations included alopecia and pale appearance (eyes, tails and ears) in Groups 2-5 throughout study. There were no indications of RBC, WBC, or Hgb changes ascribed to WINGSTAY 100 exposure. RBC size distribution width was decreased, demonstrating lack of macrocytic changes. The fertility indices (number of pregnancies/number of matings) were 79, 74, 90, 79, and 71%. Gestational indices (number of females with live litters/number of pregnancies) were 100, 93, 65, 71, and 100%, and the gestational lengths were 22.2, 22.3, 23.6, 23.8, and 23.5 days (Groups 3-5 were significantly delayed). Litter effects included stillbirths (3, 1, 45, 46, and 10% of total pups delivered), decreased pup survival (13, 13, 6, 7, and 8 live pups/litter) on post natal day 0 and 10, 10, 6, 8, and 7 on day 21. Relative liver and heart weights were increased for Groups 3-5 F1 pups. Gross observations included polycyctic kidneys in male and female F1 Groups 3-5 pups, confirmed microscopically in part as dilatation in the papillary region. Rates of these renal lesions were in excess of 80% in both male and female rats. Microscopic results for the F0 females included a 15% incidence of polycyctic kidneys in Group 5 and
none in Group 1. The other groups were not examined microscopically.

Date: 2/7/00
Test Substance: Wingstay 100 (mixed diaryl-p-phenylenediamines

Reliability: (1) valid without restriction

(14)

(1) A Photoirritation Study in Rabbits with WINGSTAY 100, Report # 3097.28 to The Goodyear Tire & Rubber Company, August 24, 1995

(2) 38-Week Accelerated Bioassay (ABA) of WINGSTAY 100 in Rats, American Health Foundation, 1996

(3) 52-Week Chronic Feeding Study of WINGSTAY 100 in Rats with 12-Week Recovery. Report # AHF R1695, American Health Foundation, 12/16/1996

(4) American Health Foundation, 32P Postlabeling Assay for Detection of Adduct Formation by WINGSTAY 100 and R-59 in Rat Liver and Urinary Bladder DNA, AHF Study No. 93-63 to The Goodyear Tire & Rubber Company, 1995

(5) American Health Foundation, Preliminary Oral 3-Week Range-Finding Study for Administration of WINGSTAY 100 and R-59 in Male and Female Fischer 344 Rats, AHF Study R-1626 to The Goodyear Tire & Rubber Company, 1994.

(6) Bayer AG Data

(7) Bayer AG, Report No. 19778, December 10, 1990.

(8) Bayer AG, Report Number 19940, February 4, 1991.

(9) Bayer AG, Unpublished Data, July 2, 1992

(10) Bioaccumulation Test of Wingstay 100 in Carp, Report Study Number 43172, Kurume Research Laboratories/CITI, 12/18/1998

(11) Four-Week Dietary Study of WINGSTAY 100 in Fischer 344 Rats, Report # AHF R1664, American Health Foundation, 1/31/96

(12) Litton Bionetics, Inc., Balb/3T3 In Vitro Transformation Assay of NAILAX, Genetics Assay No.5419 to The Goodyear Tire & Rubber Company, 1981.

(13) Litton Bionetics, Inc., Drosophilia SLRL Assay with Nailax to The Goodyear Tire & Rubber Company, 1979.

(14) Mechanistic Study of WINGSTAY 100, Report #: 65C-6429-500, Research Triangle Institute, 2/7/00

(15) Mechanistic Study of Wingstay 100, Report Study # RTI 65C-6429-500, Research Triangle Park, February 11, 2000

(16) Pharmakon USA, Report # Ph301-GY-001-93 to The Goodyear Tire & Rubber Company, 1993

(17) Pharmakon USA, Report # Ph301-GY-004-93 to The Goodyear Tire & Rubber Company, 1994

(18) Pharmakon USA, Report # Ph309-GY-001-93 to The Goodyear Tire & Rubber Company, 1993.

(19) Pharmakon USA, Report # Ph320-GY-001-93 to The Goodyear Tire & Rubber Company, 1993.

(20) Pharmakon USA, Report # Ph402-GY-001-93 to The Goodyear Tire & Rubber Company, 1993.

(22) Reseach Triangle Research, Developmental Toxicity Evaluation of WINGSTAY 100 Administered by Gavage to CD (Sprague-Dawley) Rats, Report # 65C-5962-100/200 to The Goodyear Tire & Rubber Company, July 11, 1995.

(23) Ricerca, Inc., Biodegradation Study of a Rubber Antioxidant, Document Number 6011-94-0037-BC-001 to The Goodyear Tire & Rubber Company, 1994

(24) Springborn Laboratories, A Dermal Sensitization Study in Guinea Pigs with WINGSTAY 100-Maximization Design, Report # 3097.30 to The Goodyear Tire & Rubbers Company, August 24, 1995.

(25) Springborn Laboratories, A Primary Eye Irritation Study in Rabbits with WINGSTAY 100, Report # 3097.27 to The Goodyear Tire & Rubber Company, August 24, 1995.

(26) Springborn Laboratories, A Primary Skin Irritation Study in Rabbits with WINGSTAY 100, Report # 3097.26 to The Goodyear Tire & Rubber Company, August 24, 1995.

(27) Springborn Laboratories, An Acute Toxicity Study in Rabbits with WINGSTAY 100 (Limit Test), Report # S94-001-3097.29 to The Goodyear Tire & Rubber Company, August 24, 1995.

(28) Springborn Laboratories, WINGSTAY 100-Acute Toxicity to Daphnids Under Flow-Through Conditions, Report # 96-1-6328 to The Goodyear Tire & Rubber Company, June 26, 1996.

(29) Springborn Laboratories, WINGSTAY 100-Determination of n-Octanol/Water Partition Coefficient, Report # 95-9-6103 to The Goodyear Tire & Rubber Company, December 12, 1995

(30) Springborn Laboratories, WINGSTAY 100-Prolonged (14-day) Acute Toxicity to Common Carp Under Flow-Through Conditions, Report # 96-2-6362 to The Goodyear Tire & Rubber Company, June 28, 1996

(31) Springborn Laboratories, WINGSTAY 100-Toxicity to the Freshwater Green Alga, Report # 96-4-6454 to The Goodyear Tire & Rubber Company, July 2, 1996.

(32) The Goodyear Tire & Rubber Company, Biological Effects of Nailax B in a Drosophilia melanogaster (Fruit Fly) Test System, 1979.

(33) The Goodyear Tire & Rubber Company, DNA Damage by WINGSTAY 100 Lot 48-3012 in the E. coli Pol A1- Assay, 1980.

(34) The Goodyear Tire & Rubber Company, WIGSTAY 100, Material Safety Data Sheet, 2001.

(35) The Goodyear Tire & Rubber Company, WINGSTAY 100, Material Safety Data Sheet, 2001.

(36) Two-Generation Reproductive Toxicity Evaluation of WINGSTAY 100 Administered in the Feed to CD (Sprague-Dawley) Rats, Report #: 65C-6429-400/200, Research Triangle Institute, 12/8/00.

(37) Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes on Wingstay 100 Condensation Products RWC-7703, RWX-7704, and RWC-7706, American Health Foundation, December 20, 1999

(38) WINGSTAY 100-Prolonged (14-Day) Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) Under Flow-through Conditions, Report # 96-11-6700, Springborn Laboratories, 2/21/97.

(39) Wisconsin Alumni Research Foundation, Acute Toxicity of WINGSTAY 100 to The Goodyear Tire & Rubber Company, 1959.

(40) The Sapphire Group, Derivation of Benchmark Dose from 2-Generation Rat Study,2001.

3081-01-4
1,4-Benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl-

Molecular Weight: **282.34**
Molecular Formula: **C19-H26-N2**

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance: Organic
B. Physical State: Dark purple-brown opaque liquid
C. Purity: 95-98 % Typical for Commercial Products

1.2 SYNONYMS
Santoflex® 7PPD
Santoflex® 14
p-Phenylenediamine, N-(1,4-dimethylpentyl)-N'-phenyl-

1.3 IMPURITIES
1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl (CAS# 793-24-8) <2%
4-Aminodiphenylamine (CAS# 101-54-2) <1.5%

1.4 ADDITIVES None

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	29.8°C
Decomposition:	No
Sublimation:	No
Method:	Instrumental – Differential Scanning Calorimeter, 2002
GLP:	Yes
Remarks:	Normal physical state of this material at room temperature is a slightly viscous liquid. Glass transition from –37°C to –32°C.
Reference:	Flexsys Analytical Research Report #2002.043, 2002
Reliability:	(1) Valid without restriction

*2.2 BOILING POINT

Value:	231°C
Pressure:	4.666 hPa
Decomposition:	No
Method:	Instrumental – Differential Scanning Calorimeter (DSC)
GLP:	Yes
Remarks:	Physical Constants, Flexsys SMP, R.L. Wright (1982) Pressure = 3.5 mm Hg
Reference:	L.M. Baclawski Notebook #2355311 (1982)
Reliability:	(1) Valid without restriction

†2.3 DENSITY (relative density)

Type:	Density
Value:	1.0

Temperature:	20 °C
Method:	Flexsys Standard Method of Analysis FF97.4-1
GLP:	Yes
Remarks:	Hydrometer method. Hydrometer must meet standards set in ASTM-E-100
Reference:	Flexsys 7PPD Standard Manufacturing Specifications
Reliability:	(1) Valid without restriction

***2.4 VAPOUR PRESSURE**

Value:	1.33 x 10(-10) hPa
Temperature:	25 °C
Method:	calculated Antoine Equation
GLP:	No
Remarks:	None
Reference:	Monsanto Toxicology Profile, Santoflex 14, C.E. Healy 1993
Reliability:	(2) Valid with restrictions – acceptable calculation method

***2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$**

Log Pow:	5.17
Temperature:	Not Applicable
Method:	calculated SRC LogKow (KowWin) Program 1995
GLP:	No
Remarks:	None
Reference:	Meylan, W.M. and. P.H. Howard, 1995 J. Pharm. Sci. 84: 83-92
Reliability:	(2) Valid with restrictions – acceptable calculation method

***2.6 WATER SOLUBILITY**

A. Solubility

Value:	0.67 mg/l in pH 7.0 deionized water
Temperature:	25°C
Description:	Of very low solubility
Method:	Saturated Solution/GC Analysis
GLP:	Yes
Remarks:	Preliminary solubility study for Phase I Hydrolysis
Reference:	Monsanto ABC 32305, Analytical Bio-Chemistry Labs, 1986
Reliability:	(1) Valid without restriction

B. pH Value, pKa Value

2.7 FLASH POINT

Value:	196.7 °C
Type:	Tag Open Cup
Method:	ASTM D1310, 1996
Reference:	Flexsys America Data, Test Method for Flash Points and Fire Points of Liquids by Tag Open-cup Apparatus, ASTM D1310
Reliability:	(1) Valid without restrictions

2.11 OXIDISING PROPERTIES

†2.12 OXIDATION: REDUCTION POTENTIAL

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

B. Other data - Henry's Law Constant

Results:	6.933E-011 atm-m3/mole
Remarks:	Calculated at 25°C using water solubility of 0.67 mg/l and melt point/crystallizing point of 32.4°C
Reference:	EPIWIN/HENRYWIN v3.10
Reliability:	(2) Valid with restrictions – modelling data

3. ENVIRONMENTAL FATE AND PATHWAYS

*3.1.1 PHOTODEGRADATION

Type:	Air
Indirect Photolysis:	
Type of sensitizer:	OH
Concentration of sensitizer:	156000 molecule/m3
Rate constant (radical):	227.9058E-12 cm^3/molecule-sec
Degradation:	50% after 0.563 hours
Method:	calculated
	AOP Program v1.90, 2001
GLP:	No
Test substance:	Other (calculated)
Reference:	EPIWIN/AopWin v1.90
Reliability:	(2) Valid with restrictions – accepted calculation method

*3.1.2 STABILITY IN WATER

Type:	Abiotic (hydrolysis)
Half life:	5.15 hours (calculated, not measured)
Degradation:	96% at pH 7.0 at 25 °C after 24 Hours
Method:	Extraction, ABC Protocol M-8305 (1986)
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >95%
Remarks:	Primary stock solutions of 1.00 mg/l of the test compound were prepared in nanograde acetone. Subsequent dilutions for spiking and gas chromatography standards were also prepared in nanograde acetone. Test samples were extracted with three 75ml portions of methylene chloride. The extracts were dried by passing them through a funnel containing anhydrous sodium sulfate. No test substance detected at seven days. Hydrolysis products identified by GC analysis and confirmed by GS/Mass Spectrometry as 4-hydroxydiphenylamine (35%) and Benzoquinoneimine-n-phenyl (65%). The Benzoquinoneimine-n-phenyl is the oxidized form of 4-hydroxydiphenylamine (CAS# 122-37-2, C12-H11-N-O). The amine portion of the test compound molecule was not isolated, nor was it apparent from the GC-MS spectra. It was postulated that the amine portion might be

present in the hydrolysis water layer, indicating that the linkage was cleaved at the aromatic carbon-nitrogen bond.
Reference: Monsanto ABC 32305, Analytical Bio-Chemistry Labs, 1986
Reliability: (1) Valid without restriction

***3.2 MONITORING DATA (ENVIRONMENTAL)**

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION

***3.3.1 TRANSPORT**

Type: Volatility
Media: Water
Method: Calculation from EPIWIN VP/WS 2001
Results: Volatilization half-life from model river: 1.419E+007 hours
Volatilization half-life from model lake: 1.548E+008 hours
Volatilization Constant from water: 6.93E-011 atm-m3/mole
Remarks: Model river = 1 m deep flowing at 1 m/sec and wind velocity of 3 m/sec.
Model lake = 1 m deep flowing at 0.05 m/sec and wind velocity of 0.5 m/sec.
Reference: EPISUITE/EPIWIN 2001
Reliability: (2) Valid with restrictions – modelling data

***3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)**

Media: Air-biota-sediment-soil-water
Method: Fugacity level III
Results:

	Mass Amount (%)	Half-life (hrs)	Emissions (kg/hr)
Air	0.0567	1.13	1000
Water	17.5	900	1000
Soil	51	900	1000
Sediment	31.4	3.6E+003	0

Persistence time estimated at 889 Hours
Remarks: Calculations based on user input values of water solubility of 0.67mg/l, Log Kow of 5.17, and melt/crystallizing point 32.4°C
Reference: EPISUITE/EPIWIN v3.10
Reliability: (2) Valid with restrictions – modelling data

***3.5 BIODEGRADATION**

Type: aerobic
Inoculum: adapted
Concentration of the chemical: 20.0 mg/l related to test substance
Medium: soil, raw sewage and activated sludge
Degradation: 0 % after 35 days
Results: under test condition no biodegradation observed
Kinetic
Method: ASTM Draft 3 Proposed Standard Practice for the Determination Of the Ultimate Biodegradation of Organic Chemicals (1980).
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: >95%

Remarks:	The procedure used was identical to that described in ASTM Draft #3 for Ultimate Biodegradation of Organic Chemicals. An acclimated innoculum was prepared by step-wise addition of the test compound to a defined medium over a 14-day period. The medium is derived from soil, raw sewage, and an activated sludge mixed liquor. Glucose (30.0 mg/l) was used as positive control, generating 75-93% of theory CO2 after 35 days. The theory %C for the test compound was 81.07%. Quadruplicate control flasks and triplicate flasks for the test chemical were employed.
Reference:	Monsanto ES-80-SS-48 MIC Environmental Sciences 1981
Reliability:	(1) Valid without restriction

3.6 BOD5, COD or BOD5/COD Ratio

3.7 BIOACCUMULATION

Species:	Other
BCF:	1913
Method:	BCFWIN v2.14
GLP:	No
Remarks:	Calculated using Log Pow = 5.17
Reference:	EPIWIN/BCFWIN v2.14
Relaibility:	(2) Valid with restrictions – modelling data

4. <u>ECOTOXICITY</u>

***4.1 ACUTE/PROLONGED TOXICITY TO FISH**

Type of test:	static Closed system
Species:	<u>Salmo gairdneri</u> (Rainbow Trout)
Exposure period:	96 Hours
Results:	LC_{50} (24h) = >1.00 mg/l LC_{50} (48h) = 0.70 mg/l LC_{50} (96h) = 0.42 mg/l NOEC = 0.18 mg/l LOEC = 0.32 mg/l
Analytical monitoring:	No
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >96%
Remarks:	Test fish were obtained from Spring Creek Hatchery in Lewistown, Montana. Test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations was maintained during the holding period, during which time the fish were fed a standard diet of commercial fish food until 48 hours prior to testing, when feeding was stopped. A 96-hour range-finding test preceded the definitive study. Test fish used had a mean weight of 0.73 g and a mean standard length of 36 mm. The test was conducted in 5-gallon glass vessels containing 15 liters of ABC well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 ppm and pH 8.2.

Hardness was 255 ppm and alkalinity, 368 ppm. The test vessels were kept in a water bath at 12°C. Test fish were acclimated to the dilution water and test temperature, and held without food for 48 hours prior to testing. Nanograde Acetone was used to prepare the test solutions and as the solvent control (1.0 ml). Concentrations tested were 0, 0.10, 0.18, 0.32, 0.56 and 1.0 mg/l. Fish were placed in the testing vessels within 20 minutes of the addition of the test material aliquots. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen values (7.5-8.4 mg/l, 69-78% saturation) and pH ranges (7.7-8.1) were monitored during the testing and remained within acceptable limits. As a quality check, test fish were challenged with Antimycin A. The estimated 96Hr LC50 and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.

Reference: Monsanto ABC 30687, Analytical Bio-Chemistry Labs, 1983

Reliability: (1) Valid without restriction

Type of test: static
closed-system

Species: Lepomis macrochirus (Bluegill Sunfish)

Exposure period: 96 Hours

Results: $LC_{50\ (24h)}$ = 0.38 mg/l
$LC_{50\ (48h)}$ = 0.30 mg/l
$LC_{50\ (96h)}$ = 0.30 mg/l
NOEC = 0.18 mg/l
LOEC = 0.32 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 97.9%

Remarks: Test fish were obtained from Osage Catfisheries in Osage Beach, Missouri. Test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations was maintained during the holding period, during which time the fish were fed a standard diet of commercial fish food until 48 hours prior to testing, when feeding was stopped. A 96-hour range-finding test preceded the definitive study. Test fish used had a mean weight of 0.14 g and a mean standard length of 19 mm. The test was conducted in 5-gallon glass vessels containing 15 liters of ABC well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 ppm, hardness 255 ppm, alkalinity 368 ppm, and pH 8.2. The test vessels were kept in a water bath at 22°C. Test fish were acclimated to the dilution water and test temperature, and held without food for 48 hours prior to testing. Nanograde Acetone was used to prepare the test solutions and as

the solvent control. Concentrations tested were 0, 0.1, 0.18, 0.32, 0.56 and 1.0 mg/l. Fish were placed in the testing vessels within 20 minutes of the addition of the test material aliquots. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen values (6.9-8.7 mg/l, 78-99% saturation) and pH ranges (7.8-8.1) were monitored during the testing and remained within acceptable limits. As a quality check, test fish were challenged with Antimycin A. The estimated 96Hr LC50 and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.

Reference: Monsanto ABC 30686, Analytical Bio-Chemistry Labs, 1983

Reliability: (1) Valid without restriction

Type of test: static
closed-system

Species: Pimephales promelas (Fathead Minnows)

Exposure period: 96 Hours

Results: $LC_{50\ (24h)}$ = 1.30 mg/l
$LC_{50\ (48h)}$ = 1.30 mg/l
$LC_{50\ (96h)}$ = 1.10 mg/l
NOEC = 0.32 mg/l
LOEC = 0.56 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 97.9%

Remarks: Test fish were obtained from Fattig Fish Hatchery in Brady, Nebraska. Test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations was maintained during the holding period, during which time the fish were fed a standard diet of commercial fish food until 48 hours prior to testing, when feeding was stopped. Test fish had a mean weight of 0.11 g and a mean standard length of 18 mm. The test was conducted in 5-gallon glass vessels containing 15 liters of laboratory well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 ppm, hardness (CaCO3) of 255 ppm, alkalinity of 368 ppm, and pH8.2. The test vessels were kept in a water bath at 22°C.

. Test fish were acclimated to the dilution water and test temperature, and held without food for 48 hours prior to testing. Nanograde Acetone was used to prepare the test solutions and as the solvent control (1.0 ml). Test concentrations were 0, 0.32, 0.56, 1.0, 1.8 and 3.2 mg/l for the test compound. Fish were placed in the testing vessels within 30 minutes of the addition of the test material aliquots. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved

oxygen values and pH ranges were monitored during the testing and remained within acceptable limits of 48-110% saturation (4.3-9.7 mg/l) for dissolved oxygen and pH value (8.1-8.3) consistent with control. The ammonia concentration was below the toxic limit. Water hardness (CaCO3) was 255 ppm. As a quality check, test fish were challenged with Antimycin A. The estimated 96Hr LC50 and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.

Reference: Monsanto ABC 31116, Analytical Bio-Chemistry Labs, 1983

Reliability: (1) Valid without restriction

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test: static
closed-system

Species: Daphnia magna

Exposure period: 48 Hours

Results: EC_{50} (24h) = 0.51 mg/l
EC_{50} (48h) = 0.20 mg/l
NOEC = 0.10 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: >96%

Remarks: The Daphnia magna used in the test were cultured at the ABC facilities. Adult Daphnia were fed an algae and trout chow mixture daily until 24 hours prior to testing. The bioassay was conducted in 500 ml glass beakers containing 250 ml of ABC well water. During the test, dissolved oxygen concentration ranged from 6.6-7.4 mg/l, pH range was 6.9-7.8, hardness (CaCO3) was 255 mg/l, and alkalinity was 368 mg/l. Vessels were kept in a water bath at 20°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range-finding experiment was carried out to determine the exposure concentrations for the definitive test. Acetone was used as the solvent for the test solutions, and the experiment included both a control and a solvent control (0.01ml). Concentrations (in duplicate) of the test substance were 0, 0.10, 0.18, 0.32, 0.56 and 1.0 mg/l. Ten daphnia, first instar less than 24 hours old, were placed in each test chamber. Daphnia in all concentrations were observed once every 24 hours for mortality and abnormal effects. Water quality measurements were monitored throughout the testing and were considered adequate and equivalent to those measurements in the control chamber. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program

	calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.
Reference:	Monsanto ABC 30688, Analytical Bio-Chemistry Labs, 1983
Reliability:	(1) Valid without restriction

***4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae**

Species:	Selenastrum capricornutum (freshwater alga)
Endpoint:	Biomass and Growth rate
Exposure period:	96 Hours
Results:	EC_{50} (24h) = 1.9 ppm EC50 (96h) = 0.7 ppm NOEC = 0.3 ppm LOEC = 0.6 ppm
Analytical monitoring:	No
Method:	EPA Selastrum capricornutum Printz Algal Assay Test (1978) Closed system
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >96%
Remarks:	The test algae were obtained from the US EPA Environmental Research Laboratory in Corvallis, Oregon. Beginning cell numbers in the test flasks were 1.0 x 10(4) cells/ml. Cultures were incubated at 24°C under approximately 4,300 lux illumination. Triplicate cultures were employed for each of the test concentrations and the control. Test containers were 125ml flasks containing 50ml of test medium. Concentrations for the definitive test were based on the results of a 72-hr range-finding study. These concentrations were 0, 0.3, 0.6, 1.2, 2.5 and 5.0 ppm. Reagent-grade Dimethylformamide (DMF) was used to prepare the stock solutions and as the solvent control, maximum volume 0.05 ml DMF. The pH values ranged from 7.4 at the beginning of the study, to 7.1 at the 96-hour mark. There were no other water quality measurements reported in this study. Statistical analysis involved converting each test concentration to a logarithm, and the corresponding percentage decrease of in vivo chlorophyll a or cell numbers was converted to a probit (Finny, 1971). The EC50s and 95% confidence limits were then calculated by linear regression.
Reference:	Monsanto BP-81-5-82 EG&G Bionomics, 1981
Reliability:	(2) Valid with restrictions – lack of water quality data

5. TOXICITY

***5.1 ACUTE TOXICITY**

5.1.1 ACUTE ORAL TOXICITY

Type:	LD_{50}
Species/strain:	Rats, Sprague-Dawley Albino
Value:	2100 mg/kg bw
Sex:	Male and female
# of Animals:	25
Vehicle:	None - undiluted
Doses:	1260, 1580, 2000, 2510 or 3160 mg/kg bw

Method: Single Oral Dose, Younger Laboratories Protocol, 1973
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >96 %
Remarks: Five groups of male and female rats (5 animals/dose level) were fed a single oral dose of the undiluted test article via oral gavage. Male rats had initial average body weights of 210-240 grams: females had initial average body weights of 215-235 grams. Dosages were 1260, 1580, 2000, 2510 and 3160 mg/kg. Clinical signs of toxicity included reduced activity and appetite for 2-4 days for survivors, and increasing weakness, collapse and death for decedents in 1-4 days. Gross autopsy findings on decedents were hemorragic areas in the lungs, discolored livers and acute gastrointestinal inflammation. Survivors were sacrificed after seven days. All viscera of survivors appeared normal. 95% confidence limits: 2000-2200 mg/kg.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
1260	0/3	1/2	1/5
1580	0/2	2/3	2/5
2000	0/3	2/2	2/5
2510	2/2	3/3	3/5
3160	2/3	2/2	4/5

Reference: Monsanto Y-73-169 Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.1.2 ACUTE INHALATION TOXICITY

Type: LC_{50}
Species/strain: Rats, Sprague-Dawley Albino
Exposure time: 6 Hours
Sex: Male
# of Animals: 6
Value: >0.14 mg/kg
Method: Acute Inhalation LC50, Younger Laboratories Protocol, 1967
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >95%
Remarks: Six male rats were exposed to the test article at a concentration of 0.14 mg/l in warmed (76.5°F) air for 6 hours. All animals survived. After a 10-day recovery period, all animals were sacrificed. No clinical signs of toxicity were noted and all viscera appeared normal.
Reference: Monsanto Y-67-101, Younger Laboratories, 1967
Reliability: (2) Valid with restrictions – age of study, lack of method detail

Type: LC_{50}
Species/strain: Rats, Sprague-Dawley Albino
Exposure time: 6 Hours
Sex: Male
# of Animals: 6
Value: Not determined - No vaporization at room temperature
Method: Acute Inhalation LC50, Younger Laboratories Protocol, 1973
GLP: No data

Test substance: As prescribed by 1.1-1.4, purity: >95%
Remarks: Six male rats were exposed to the test article in a stream of ambient air for 6 hours. All animals survived. The initial sample weight of the test substance was 134.0 grams, as was the final weight after six hours, indicating no volatility at normal room temperatures. After an uneventful 10-day observation period, all animals were sacrificed. No clinical signs of toxicity were noted and all viscera appeared normal.
Reference: Monsanto Y-73-169, Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.1.3 ACUTE DERMAL TOXICITY

Type: LD_{50}
Species/strain: Rabbits, New Zealand Albino
Exposure time: 24 Hours
Sex: Male and female
# of Animals: 3
Vehicle: None - undiluted
Value: >5010 mg/kg bw
Method: Single Dermal Dose, Younger Laboratories Protocol, 1973
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >95%
Remarks: The undiluted test article was applied to the shaved skin of two male and one female rabbits at dose levels of 5010 or 7940 mg/kg bw. Males in this study weighed 2.2 and 2.5 kg initially, and the female weighed 2.4 kg. The test material was held in place by means of an occlusive wrap of latex rubber and secured by bandaging and elastic tape. The occlusive wrap was removed after 24 hours and the excess material was wiped from the test animal. Clinical observations were made three times during the first eight hours after dosing, and twice daily thereafter until sacrifice. Clinical signs of toxicity noted were reduced appetite and activity for 4-7 days in survivors, and increased weakness, collapse and death at 8 days for decedents. Gross autopsy findings in decedents included hemorragic areas in the lung, liver and spleen, and discoloration of the kidneys. General gastrointestinal inflammation was also noted. Survivors were sacrificed after 14 days. All viscera in survivors appeared normal.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
5010	0/1	----	0/1
7940	1/1	0/1	1/2

Reference: Monsanto Y-73-169 Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.2.1 SKIN IRRITATION/CORROSION

Species/Strain: Rabbits, New Zealand Albino
Sex: Male and female
# of Animals: 6
Vehicle: None - undiluted
Value: 0.0/8.0

Results:	Not Irritating
Classification:	Non-Irritating
Exposure Time:	24 Hours
Method:	Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: >96%
Remarks:	0.5 ml of the undiluted test substance was applied to the shaved dorsal areas of six albino rabbits. The test material was applied to the skin under 1" square gauze patches and held in contact with the skin by means of an occlusive wrap of latex rubber secured by bandaging and elastic tape. The occlusive wrap and gauze patches were removed after 24 hours. Dermal irritation was scored by the Draize Method, and results were recorded 24, 48, 72 and 168 hours after topical application. The Primary Irritation Index was calculated by averaging the mean scores at 24 and 72 hours. The Primary Irritation Index was found to be 0.0 on a scale of 0.0-8.0. A slight defatting effect was noted, with skin flaking off in 7-10 days. There was no injury noted in depth.
Reference:	Monsanto Y-75-78 Younger Laboratories May 7, 1975
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

5.2.2 EYE IRRITATION/CORROSION

Species/strain:	Rabbits, New Zealand Albino
Sex:	Male and female
# of Animals:	6
Vehicle:	None - undiluted
Value:	3.5/110.0
Results:	Slightly irritating
Classification:	Non-irritating
Exposure Time:	24 Hours
Method:	Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP:	No data
Test substance:	As prescribed in 1.1-1.4, purity: >96%
Remarks:	0.1 ml of the undiluted test substance was applied to one eye of six albino rabbits. The other eye was not treated and served as a control. The cornea, iris and conjuntivea were examined immediately after treatment, and then at intervals of 1 hour, and at 24, 48, 72 and 168 hours. The Draize Method was used for scoring eye irritation. Immediate findings: slight discomfort. At 1 hour: slight erythema, very slight edema, copious discharge At 24 hours: slight erythema, moderate to copious discharge At 48 hours: slight erythema, slight discharge At 72 hours: all animals scored "0" The average Draize score for 24, 48 and 72 hours was calculated for each animal and then averaged over the six animals. The average Draize score was 3.5 on a scale from 0-110.
Reference:	Monsanto Y-75-78 Younger Laboratories May 7, 1975
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

*5.4 REPEATED DOSE TOXICITY

Species/strain: Rats, Sprague-Dawley Albino
Sex: Male/Female
# of Animals: 50 (25 male, 25 female, 5/sex/dose)
Route of Administration: Oral feed
Exposure period: 28 days
Frequency of treatment: Daily
Post exposure observation period:
Dose: 0, 500, 750, 1500 and 3000 ppm
Control group: Yes
Concurrent vehicle
NOEL: 500 ppm
LOEL: 750 ppm
Results: The test article was administered to groups of 25 male and 25 female rats in a controlled study for one month. The test rats, approximately seven weeks old, had starting weight ranges of 230.1-278.9 grams for males, and 157.9-185.1 for females. Verification of test article stability and dose levels was analyzed and confirmed via gas chromatography. Animals were observed twice daily and weighed weekly. Overall averages for dietary concentrations were established as 0, 450, 660, 1300 and 2800 ppm. The animals were checked twice daily for mortality and moribundity. Detailed observations for toxicity were performed once weekly, as were body weight and food consumption measurements. A gross pathology examination was performed on all animals at terminal sacrifice. Animals were examined internally and externally, internal cavities were opened, organs were examined in place and then removed. Hollow organs were opened and examined, and lever weights were recorded. There were no mortalities during the in-life portion of the study. Toxicity during the in-life phase was indicated by a dose-related reduction of food intake and reduced body weight gains in both males and females at all dietary levels. There were no clinical signs of toxicity observed during the study. There were no gross pathology changes noted at sacrifice which were considered treatment-related, and no significant differences in liver weights or organ coloration. The NOEL for male rats was considered to be 500 ppm. The same NOEL was marginally established for female rats, even though there was a slight, but not statistically significant difference seen in average body weights.
Method: OECD Guidelines for Testing of Chemicals, Section 412, 1981
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96.2%
Reference: Monsanto ML-87-309, Environmental Health Lab, 1987
Reliability: (1) Valid without restriction

*5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

Type: Bacterial Reverse Mutation Assay - Ames
System of testing: Salmonella typhimurium TA-1535 TA-1537 TA-1538 TA-98 TA-100
Concentration: 0.001, 0.01, 0.1, 1.0 and 5.0 microliters/plate
Metabolic activation: With and without

Results:
Cytotoxicity conc: With metabolic activation: 5.0 ul/plate (TA-98 only)
Without metabolic activation: 5.0 ul/plate (TA-98 only)
Precipitation conc: Not Determined
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Ames Mutagenicity Plate Test (Overlay Method) 1975
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96.2%
Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were methylnitrosoguanidine (MNNG), 2-nitrofluorene (NF) and quinacrine mustard (QM). Positive control chemicals used for the activation assays were 2-anthramine (ANTH), 2-acetylaminofluorine (AAF) and 8-aminoquinoline (AMQ). Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Statistical analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.
Reference: Monsanto BIO-76-229, Litton Bionetics, 1976
Reliability: (1) Valid without restriction

B. NON-BACTERIAL IN VITRO TEST

Type: Mitotic Recombination Assay
System of testing: Saccharomyces cerevisiae, D4
Concentration: 0.001, 0.01, 0.1, 1.0 and 5.0 microliters/plate
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: None
Without metabolic activation. None
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Ames Mutagenicity Plate Test (Overlay Method) 1975
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >96 %
Remarks: The test compound was evaluated for genetic activity in assays with and without the addition of mammalian metabolic activation preparations. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-

Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. The chemical used as the positive control for the non-activation assay was methylnitrosoguanidine (MNNG) at 10 ug/plate. Positive control chemical used for the activation assay was DMNA at 100 micromoles/plate. Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Statistical analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: Monsanto BIO-76-229, Litton Bionetics, 1976
Reliability: (1) Valid without restriction

Type: Forward Mutation Mouse Lymphoma Assay
System of testing: L5178Y Mouse Lymphoma Cells
Concentration: 0.625 – 10.0 nl/ml without activation (duplicate)
1.25 – 50.0 nl/ml with activation (duplicate)
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 60 nl/ml
Without metabolic activation: 20 nl/ml
Precipitation conc: Not Determined
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Clive, D., and Spector, J.F.S., Laboratory Procedure for Assessing Specific Locus Mutations at the TK Locus in Cultured L5178Y Mouse Lymphoma Cells. Mutation Res., 31:17-29, 1975
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: >96%
Remarks: The test substance was dissolved in DMSO at 500 ul/ml. Stock solutions in DMSO were diluted 1:100 into growth medium to give applied doses ranging from 5 ul/ml to 0.039 nl/ml. DMSO (1%) was used as the solvent control substance. Growth medium without the addition of solvent was used as a negative control. No genetic effects were attributed to the presence of the solvent. The activation system was S9, prepared from the livers of Aroclor 1254-induced male Fischer 244 rats. Ethylmethane sulfonate (EMS, 0.5 ul/ml, non-activation studies) and Dimethylnitrosamine (DMN, 0.3 ul/ml, activation studies) were used as reference mutagens and induced mutation frequencies within the expected range.

	Conc.	Mutant clones	Viable clones	Mutant frequency x10E-6
Non-Activation				
Solvent Control	---	50.0	413.0	12.1
Negative Control	---	53.0	293.0	18.1
EMS	0.5	562.0	82.0	685.4

Test Compound	0.625	48.0	258.0	18.6
	1.250	46.0	247.0	18.6
	2.500	48.0	186.0	25.8
	5.000	56.0	221.0	25.3
	10.000	56.0	233.0	24.0
Activation with S-9				
Solvent Control	---	50.0	257.0	19.5
Negative Control	---	60.0	233.0	25.8
DMN	0.3	65.0	5.0	1300.0
Test Compound	1.250	67.0	277.0	24.2
	2.500	60.0	204.0	29.4
	5.000	69.0	295.0	23.4
	10.000	99.0	290.0	34.1
	20.000	29.0	195.4	8.6
	30.000	80.0	267.0	30.0
	40.000	54.0	113.0	47.8
	50.000	93.0	296.0	31.4

Reference: Monsanto BO-78-225, Litton Bionetics, 1979
Reliability: (1) Valid without restriction

Type: Forward Gene Mutation Assay, CHO/HGPRT
System of testing: Chinese Hamster Ovary cells, K1BH4
Concentration: 0, 1, 3, 5, 7 and 10 ug/ml without activation (triplicate)
0, 10, 15, 20, 25 and 30 ug/ml with activation (triplicate)
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 7 ug/ml
Without metabolic activation: 5 ug/ml
Precipitation conc: Not Determined
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: CHO/HGPRT Mutation Assay (1981) Hsie, et.al.
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96.2%
Remarks: The subclone K1BH4 CHO cells were obtained from Dr. Hsie at the Oak Ridge National Laboratory. The test material was stored refrigerated and protected from light exposure as recommended. Solutions of the test material were prepared using Acetone as solvent on the day of treatment. The positive controls used were benzo(a)pyrene for the activation assay, and ethylmethane sulfonate (EMS) for the non-activated assay. The exogenous activation system was Aroclor 1254 induced rat liver homogenate (S9), Mutagenicity data were analyzed according to the statistical method of Snee and Irr (1981) designed specifically for the CHO/HGPRT mutation assay. Student's t-test was used to compare treatment data to solvent data. A range-finding experiment to determine the cytotoxicity of the test compound preceded the mutagenicity experiments. Test compound concentrations of 0, 0.3, 0.7, 1.0, 3.0, 7.0, 10, 30, 70, 100 and 300 ug/ml with 1%, 2%, 5% and 10% S9 were used in this preliminary experiment. Because none of the treatments

performed with the various S9 concnetrations yeilded a statistically significant response, a concentration of 5% S9 was chosen for the confirmation experiment. (This level has been shown to provide significant mutagenic responses when tested with a wide variety of promutagens used in this assay (A.P. Li, 1984). No statistically significant increases in mutation frequency were observed in two separate experiments in any of the treated cultures in the presence or absence of S9 activation at any level tested. The test compound was not mutagenic in CHO cells under these experimental comditions.

Reference: Monsanto ML-87-340, Environmental Health Labs, 1988
Reliability: (1) Valid without restriction

Type: In vitro Cytogenetics Study
System of testing: Chinese Hamster Ovary (CHO) cells
Concentration: 0, 1.5, 5, 7.5, 10.0 and 15.0 ug/ml (duplicate)
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 12.5 ug/ml
Without metabolic activation: 12.5 ug/ml
Precipitation conc: Not Determined
Genotoxic effects:
With metabolic activation: Weak Positive
Without metabolic activation: Weak Positive
Method: Preston, Et. al., Mammalian In vivo and In vitro Cytogenics Assays: A report to the U.S. Gene-Tox Program (1981)
GLP: Yes
Test substance: . As prescribed by 1.1-1.4, purity: 96.2%
Remarks: Treatment solutions were made using Acetone. Two range-Finding experiments were run to determine the optimum dose concentrations. The exogenous activation system was Aroclor 1254-induced rat liver homogenate (S9). MMS and CP were used as concurrent positive controls for treatment with and without S9 activation, respectively. Duplicate samples per treatment condition were used. Chi-square analysis was used to analyze the number of cells with structural aberrations. Dunnett's t-test was used to analyze structural aberrations per cell. Scoring for cytogenetic damage was performed on the solvent controls, positive controls, and the three highest dose levels of the test chemical. The cells were scored for both mitotic index and average cell generation time and compared to the solvent control.
Average cell generation time was 12 hours for both, with a mitotic index of 5-8%. Statistically significant increases in number of cells with structural aberrations and average structural aberrations/cell were observed at the 15 ug/ml level for the 48 hour harvest time and for average structural aberrations/cell at the 24 hour harvest time without S9 activation. A significant dose-response was not observed. The aberrant cells harvested at 24 and 48 hours included mainly cells with chromatid- and chromosome-type deletions, with a few decentrics and cells with chromatid interchanges. This was also observed in the solvent control. The
positive MMS control yielded significant increases in both cells with structural aberrations and number of aberrations/cell. With

S9 activation, a statistically significant increase in the number of cells with structural aberrations, and number of aberrations/cell was observed at the 10 ug/ml dose level, and for the number of aberrations/cell at 7.5 ug/ml and 12 hour harvest time. No dose-related response was observed. Aberrations were mainly deletions, with a few cells having chromatid interchanges, intrachanges and triradials. The positive control yielded the expected positive response. A retest confirmed results. It was concluded that the test compound exhibited weak clastogenicity in CHO cells under these experimental conditions.

Reference: Monsanto ML-87-341, Environmental Health Labs, 1989
Reliability: (1) Valid without restriction

*** 5.6 GENETIC TOXICITY IN VIVO**

Type: Mammalian Bone Marrow Metaphase Assay
Species/strain: Rats, Sprague-Dawley
Sex: Male/Female
Route of Administration: Oral gavage
Exposure period: 6, 18 and 30 hours
Doses: 1100 mg/kg/bw (slightly above ½ the oral LD50)
Results:
Effect on mitotic index or P/N ratio: None
Genotoxic effects: Negative
Method: Preston, et al., Mammalian In vivo and In vitro Cytogenics Assays: A Report to the U.S. Gene-Tox Program (1981)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity:96.2%
Remarks: Groups of male and female rats (5/sex/dose level) were dosed with 1050, 1100, 1200, 1500 and 2000 mg/kg/bw in two range-finding studies. Based upon the results, a dose level of 1100 mg/kg/bw was chosen as close to the maximum tolerated dose for the metaphase analysis. The positive control chemical, Cyclophosphamide (CP) was administered to the positive control animals at 18 hours at 20 mg/kg bw by oral gavage. The vehicle control, deionized water, was administered at 5 ml/kg bw at 6, 18 and 30 hours. During the In vivo phase, test animals were observed for pharmacotoxicity immediately after dosing, and at 6, 18 and 30 hours. Observations indicated moderate to severe pharmacotoxic signs. Two to three hours prior to sacrifice, each animal received a single intraperitoneal dose of colchicine at 4 mg/kg bw to arrest dividing cells in metaphase. Both femurs were removed from each animal after sacrifice. The distal end was snipped off one bone and the proximal end off the other. Bone marrow cells were flushed, washed and centrifuged, and slides were prepared using freshly prepared fixative. A total of 500 well-spread metaphase cells with a minimum of overlapping chromosomes were scored for the presence of chromosome aberration per experimental treatment point (50 per animal) by two investigators (25 each per animal). Cells judged acceptable for analysis based on cell morphology and total chromosome number were further analyzed with 100x oil immersion objective

where abnormalities were detected and classified. The mean number of aberrations per cell per animal was analyzed for statistically significant increases by one-tailed t tests for each time interval. The test compound did not produce significant increases in the number of aberrations or in the number of aberrant metaphases at any of the three sacrifice times evaluated. Pharmacotoxic signs observed during the study indicated that the test chemical was dosed near the maximum tolerated dose. Conclusion was that the test chemical was negative in ability to induce structural chromosomal aberrations to the hemopoietic cells of the rat bone marrow under test conditions.

Compound	Dose	Harvest time	# rats	# metaphazes analyzed	Aberrations/group
DI water	5 ml/kg	6hr	10	500	2
Test Cpd.	1100 mg/kg	6hr	10	500	2
DI water	5 ml/kg	18hr	10	500	6
Test Cpd.	1100 mg/kg	18hr	10	500	6
CP	20 mg/kg	18hr	10	500	644
DI water	5 mg/kg	30hr	10	500	1
Test Cpd.	1100 mg/kg	30hr	10	500	5

Reference: Monsanto PK-88-342, Pharmakon Research, 1988
Reliability: (1) Valid without restriction

***5.8 TOXICITY TO REPRODUCTION**

***5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY**

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

Type: Immunotoxicity – Repeat Insult Patch Test
Human skin, Santoflex 14 Antiozonant
Shelansky Method (Procedings of the Toilet Goods Association, No. 19, May 1953)

Results: Fifty human volunteers not previously exposed to test rubber formulations were selected. Squares soaked in the test material were applied to the arm or back and held in place with tape. Patches were removed after 24 hours and the sites examined for reactions, after which the material was reapplied. Fifteen such primary applications were made, followed by a 2-week rest period. A challenge application was then applied as before, and to the same site. No reactions were produced by either the primary or challenge applications. There was no evidence of primary irritation or skin fatigue. There was no evidence of skin sensitization under the test conditions.

Remarks: Concentration of test article was not noted. Both male and female volunteers were used in the study.

Reference: Monsanto SH-65-3, Industrial Biology Labs, 1965
Reliability: (2) Valid with restrictions – age of study, lack of method detail

Type:	Immunotoxicity – Repeat Insult Patch Test Human skin, Unvulcanized Rubber containing Santoflex 14 Antiozonant Shelansky Method (Procedings of the Toilet Goods Association, No. 19, May 1953)
Results:	Fifty one human volunteers not previously exposed to test rubber formulations were selected. The test material, in the form of 1" squares of unvulcanized rubber, was affixed to the upper arm of each test subject and covered with gauze (occluded). Patches were removed after 24 hours and the sites examined for reactions. Direct effects by single contact were graded with a numerical score ranging from 0 (no response) to 4 (severe response) for primary irritation. Choice of contact site for the second and all subsequent applications was based on the condition of the skin at the original contact site. If irritation occurred, a different site was chosen. If no irritation occurred, the test patch was reapplied to the same site. There were 15 such applications in the induction phase of the study. Following a 14-day rest period, a challenge application was applied at the original contact site. No visible skin changes were noted on any test subject during either the induction phase or the challenge phase of the study. The test article was considered to be negative for primary skin irritation, negative for skin fatigue by sequential contact, and negative for delayed contact hypersensitivity.
Remarks:	Concentration of test article in the rubber compound was 3 parts per 100 parts of SBR 1000 rubber (3 phr) Both males and females were used in the study.
Reference:	Monsanto SH-67-13, Industrial Biology Labs, 1967
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

6. REFERENCES

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12. EPISUITE/EPIWIN v3.10

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14. EPIWIN/BCFWIN v2.14

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28. Monsanto SH-67-13, Repeated Insult Patch Test using Unvulcanized Rubber Sheets, Industrial Biology Laboratories, Inc., January 15, 1968

29. Monsanto Experiment No. 49-48, Stocks for Dermatitis Studies Batch Sheet, B-1 Masterbatch for SH-67-13, 1967

I U C L I D

D a t a S e t

Existing Chemical	ID: 15233-47-3
CAS No.	15233-47-3
TSCA Name	1,4-benzenediamine, N-(1-methylheptyl)-N'-phenyl-
EINECS No.	239-281-1
Molecular Weight	296

Producer Related Part	
Company:	Bayer Corporation
Creation date:	08-NOV-2001

Substance Related Part	
Company:	Bayer Corporation
Creation date:	08-NOV-2001

Memo:	RAPA PPD Category

Printing date:	09-NOV-2001
Revision date:	
Date of last Update:	09-NOV-2001

Number of Pages:	19

Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 OECD and Company Information

Type: lead organisation
Name: American Chemistry Council (formerly Chemical Manufacturers Association) Rubber and Plastics Additives (RAPA) HPV Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States
Phone: 703-741-5600
Telefax: 703-741-6091

08-NOV-2001

Type: cooperating company
Name: Bayer Corporation
Country: United States

08-NOV-2001

Type: cooperating company
Name: Ciba Specialty Chemicals Corporation
Country: United States

08-NOV-2001

Type: cooperating company
Name: Crompton Corporation
Country: United States

08-NOV-2001

Type: cooperating company
Name: Flexsys America L.P.
Country: United States

08-NOV-2001

Type: cooperating company
Name: Noveon, Inc (formerly BF Goodrich)
Country: United States

08-NOV-2001

Type: cooperating company
Name: R.T. Vanderbilt Company, Inc.
Country: United States

08-NOV-2001

Type: cooperating company
Name: The Goodyear Tire & Rubber Company
Country: United States

08-NOV-2001

Type: cooperating company
Name: The Lubrizol Corporation
Country: United States

08-NOV-2001

Type: cooperating company
Name: UOP, LLC.
Country: United States

08-NOV-2001

1.0.2 Location of Production Site
-

1.0.3 Identity of Recipients
-

1.1 General Substance Information

Substance type: organic
Physical status: liquid
Purity: > 95 % w/w
08-NOV-2001

1.1.0 Details on Template
-

1.1.1 Spectra
-

1.2 Synonyms

N-phenyl - N'-(1-methylhepyl)-p-phenylenediamine
08-NOV-2001

UOP 688 Antiozonant
08-NOV-2001

1.3 Impurities
-

1.4 Additives
-

1.5 Quantity
-

1.6.1 Labelling
-

1.6.2 Classification
-

1.7 Use Pattern
-

1.7.1 Technology Production/Use
-

1.8 Occupational Exposure Limit Values
-

1.9 Source of Exposure
-

1.10.1 Recommendations/Precautionary Measures
-

1.10.2 Emergency Measures
-

1.11 Packaging
-

1.12 Possib. of Rendering Subst. Harmless
-

1.13 Statements Concerning Waste
-

1.14.1 Water Pollution
-

1.14.2 Major Accident Hazards
-

1.14.3 Air Pollution
-

1.15 Additional Remarks
-

1.16 Last Literature Search
-

1.17 Reviews
-

1.18 Listings e.g. Chemical Inventories
-

2.1 Melting Point

Value:
Remark: Unknown, no studies available
08-NOV-2001

2.2 Boiling Point

Value: 431 degree C at 1013 hPa
Method: other: no data
GLP: no
08-NOV-2001 (1)

2.3 Density

Type: relative density
Value: 1.003 at 15.6 degree C
Method: other: no data
GLP: no
Result: Specific gravity = 1.003
08-NOV-2001 (1)

2.3.1 Granulometry
-

2.4 Vapour Pressure

Value:
Remark: Unknown, no studies available
08-NOV-2001

2.5 Partition Coefficient

log Pow:
Method: OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year:
Result: Method not applicable.
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
08-NOV-2001 (2)

2.6.1 Water Solubility

Qualitative: not soluble
Method: OECD Guide-line 105 "Water Solubility"
Remark: Evaluation as part of Certificate of Analysis
Result: Insoluble;
pH Value, pKa Value: Unknown, no studies available
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
08-NOV-2001 (2)

2.6.2 Surface Tension
-

2.7 Flash Point
-

2.8 Auto Flammability
-

2.9 Flammability
-

2.10 Explosive Properties
-

2.11 Oxidizing Properties

Result:
Remark: Unknown, no studies available
08-NOV-2001

2.12 Additional Remarks

Memo: Fat Solubility
Method: OECD 116
Result: 100%
08-NOV-2001 (2)

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000 molecule/cm3
Rate constant: .000000000229 cm3/(molecule * sec)
Degradation: 50 % after .6 hour(s)
Method: other (calculated): AOP Program (v1.89)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

3.1.2 Stability in Water
-

3.1.3 Stability in Soil
-

3.2 Monitoring Data (Environment)
-

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
Media: other: air - water - soil - sediment
Air (Level I):
Water (Level I):
Soil (Level I):
Biota (L.II/III):
Soil (L.II/III):
Method: other: EPIWIN, Level III Fugacity Model
Year: 1999
Result:

Media	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	0.0248	1.12	1000	7.34e-013
Water	8.94	900	1000	2.61e-014
Soil	43.4	900	1000	3.56e-016
Sediment	47.6	3.6e+003	0	1.76e-014

Media	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	615	9.94	20.5	0.331
Water	275	358	9.18	11.9
Soil	1.34e+003	0	44.6	0
Sediment	367	38.1	12.2	1.27

Persistence Time: 1.33e+003 hr

Reaction Time: 1.54e+003 hr
Advection Time: 9.86e+003 hr
Percent Reacted: 86.5
Percent Advected: 13.5
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

3.3.2 Distribution
-

3.4 Mode of Degradation in Actual Use
-

3.5 Biodegradation
-

3.6 BOD5, COD or BOD5/COD Ratio
-

3.7 Bioaccumulation
-

3.8 Additional Remarks
-

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other
Species: other: Freshwater fish
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: .067
Method: other: ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: Chemical may not be soluble enough to measure this predicted effect.
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

Type: other
Species: other: Saltwater fish
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: .094
Method: other: ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: Chemical may not be soluble enough to measure this predicted effect.
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: other
Species: Daphnia sp. (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : .093
Method: other: ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: Chemical may not be soluble enough to measure this predicted effect.
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

Type: other
Species: Mysidopsis bahia (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50 : .00134
Method: other: ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Reliability: (2) valid with restrictions
Acceted calculation method
08-NOV-2001 (3)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green algae
Endpoint:
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: .072
Method: other: ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: Chemical may not be soluble enough to measure this predicted effect.
Reliability: (2) valid with restrictions
Acceted calculation method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (3)

4.4 Toxicity to Microorganisms e.g. Bacteria
-

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish
-

4.5.2 Chronic Toxicity to Aquatic Invertebrates
-

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms
-

4.6.2 Toxicity to Terrestrial Plants
-

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species
-

4.7 Biological Effects Monitoring
-

4.8 Biotransformation and Kinetics
-

4.9 Additional Remarks
-

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type:	LD50
Species:	rat
Strain:	other: Holtzman
Sex:	male
Number of Animals:	5
Vehicle:	other: corn oil
Value:	4.3 mg/kg bw
Method:	other: Method described by Weil, C.S., Biometrics 8, 249, 1952
Year:	1952 GLP: no
Test substance:	other TS: Commercial product, >95% purity
Method:	UOP 688 was administered orally to six groups , each composed of 5 male albino rats, weight range 219-251 grams. Each dose was administered either undiluted or as a 10% volume/volume solution in corn (Mazola) oil. Dosage levels tested were 0.046, 0.10, 2.15, 4.46, 10.0, and 21.5 mg/kg body weight. All animals were observed closely for gross signs of systemic toxicity and mortality during the day of dosage, and at least once daily thereafter for 14 days. All animals were subject to gross necropsy at study termination.
Result:	Animals in the 0.046, 0.1, and 2.15 mg/kg dosage levels generally exhibited normal appearance and behaviour throughout the 14 day period. Rats at the 4.64 mg/kg dose level began showing depression, slowed righting reflexes, and diarrhea on the second day following dosage. On the fourth day after dosage, one rat showed labored respiration, ataxia, depressed righting, placement, and pain reflexes, and a marked bloody nasal discharge. These signs generally continued until death occurred, or until the fifth day following dosage when the two surviving rats appeared normal. The rats in the 10.0 and 21.5 mg/kg doe levels showed diarrhea, unkempt fur, depression, depressed relexes, and a dark oily stain in the perineal area on the day after dosage. These signs continued until death occurred. Death was preceded by lacrimation and coma.
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag:	Critical study for SIDS endpoint

08-NOV-2001 (4)

5.1.2 Acute Inhalation Toxicity

Type:
Species:
Strain:
Sex:
Number of
Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: GLP:
Test substance:
Remark: Unknown, no studies available.
Not an appropriate route of exposure due high boiling point.
08-NOV-2001

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: New Zealand white
Sex: male/female
Number of
Animals: 10
Vehicle:
Value: > 2000 mg/kg bw
Method: other: U.S. Code of Federal Regulations 40 CFR 163
Year: GLP:
Test substance: other TS: Commercial product, >95% purity
Method: The test material was applied to five male and five female white New Zealand white rabbits. The dose was applied to the abdominal skin which had been previously been shaven. The abdominal skin area of all the rabbits was abraded by making a series of longitudinal minor epidermal incisions placed two to three centimeters apart, using a hypodermic needle as a cutting tool. The abrasions were sufficiently deep to penetrate the epidermis, but not to induce bleeding. The undiluted sample was applied at a dosage level of 2.0 grams/kg of body weight. The test sample was kept in contact with the skin on at least 10% of the body surface. During the exposure period, each rabbit was observed for signs of toxicity at two, four and five and one half hours post application. After 23 ¾ to 24 hours of skin contact exposure, any unabsorbed sample remaining on the skin was removed by gentle sponging with a moistened towel. Rabbits were observed for 14 days following completion of the exposure period. Examinations for gross signs of systemic toxicity were carried out twice daily during this period. At the end of the 14 day observation period, rabbits were weighted, sacrificed and gross necropsy was performed.
Remark: study reviewed by lab QA Director
Result: One female rabbit was found dead on day two. Necropsy

revealed diarrhea stains around the anus, congested lungs, a mottled and darkened liver, stomach and intestine which appeared autolytic and pale but congested kidneys. Erythemia and edema followed by desquamation and atonia were seen at the application site in all surviving animals. Four rabbits exhibited spotted whitening on the day of exposure completion. Systemic effects were limited to transient nasal discharge in two animals and transient green colored urine in one animal.
Reliability: (1) valid without restriction
Meets National standards method
Flag: Critical study for SIDS endpoint
08-NOV-2001 (5)

5.1.4 Acute Toxicity, other Routes
-

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration:

Exposure: Semiocclusive
Exposure Time: 24 hour(s)
Number of Animals: 6
PDII: 1.5
Result:
EC classificat.:
Method: other: U.S. Code of Federal Regulations 40 CRF 163
Year: GLP:
Test substance: other TS: Commercial product, >95% purity
Method: 0.5 ml undiluted test material was applied under one inch square surgical gauze patches to two abraded skin areas and two intact skin areas on each of six New Zealand White rabbits. After 24 hours of skin contact exposure, any unabsorbed sample remaining on the skin was removed by gentle sponging with a moistened towel. The reactions were scored immediately after removal of the patches (24 hour reading), and again two days later (72 hour reading).
Remark: study reviewed by lab QA Director
Result: Irritative effects noted during the course of the study included very slight to well defined erythema, at the abraded and intact sites of all animals. Very slight to slight edema scores were noted in five animals on the abraded and intact sites. The Primary Irritation Index was found to be 1.5. Some loss of skin resiliency (atonicity) was noted. No evidence of corrosivity was observed.
Reliability: (1) valid without restriction
Meets National standards method
09-NOV-2001 (5)

Species: rabbit
Concentration: undiluted

Exposure: Semiocclusive
Exposure Time:
Number of
Animals: 6
PDII:
Result:
EC classificat.:
Method: other: U.S. Code of Federal Regulations 49 CFR 173.136 -137
Year: 1992 GLP: yes
Test substance: other TS: Commercial product, Lot #0483, >95% purity
Method: The primary dermal irritation/corrosivity potential was evaluated when applied to the skin of 3 male and 3 female rabbits under 3 minute, 1 hour, and 4 hour semi-occluded conditions. Each application site was examined for erythemia and edema according to the Draize method.
Result: No evidence of corrosion was observed at any of the test sites for any of the exposure periods.

Not considered corrosive to the skin of rabbits
Reliability: (1) valid without restriction
GLP Guideline study
09-NOV-2001 (6)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time:
Comment: other: see method
Number of
Animals: 9
Result:
EC classificat.:
Method: other: U.S. Code of Federal Regulations 40 CFR 163
Year: GLP:
Test substance: other TS: Commercial product, >95% purity
Method: 0.1 ml of the undiluted test material was applied to the left or right eye of each of nine rabbits. The opposite eye served as a control. The treated eyes of six rabbits were left unrinsed. The treated eye of three rabbits were rinsed after 30 seconds for 60 seconds with 200 ml of lukewarm water. Examinations for gross signs of eye irritation were made approximately 24, 43, and 70 ½ hours and four, seven, ten, thirteen, sixteen, and nineteen days following application. Scoring of irritative effects was according to the method of Draize.
Remark: study reviewed by lab QA Director
Result: Non-rinsed eyes - Irritative effects noted during the study included isolated occurrences of mild corneal opacity with up to one-quarter of the corneal area involved in the two

rabbits. Conjuctival effects included isolated occurrences of mild erythema in five rabbits. Total irritation score ranged from 0-5.

Rinsed eyes - Mild corneal irritation was observed in the rinsed eye group. These effects generally cleared after four days post-treatment with opacity occurring once after this reading in one rabbit. Sporadic occurrences of mild to moderate conjunctival irritation on days 13 and 19 were noted in three rabbits. The total irritation scores ranged from 0-7.

09-NOV-2001 (5)

5.3 Sensitization

Type:	Patch-Test
Species:	human
Number of Animals:	15
Vehicle:	other: acetone
Result:	not sensitizing
Classification:	not sensitizing
Method:	other: Adapted from the repeated insult patch test procedure described by Draize (Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, pp. 52-55, The Association of Food and Drug Officials of the United States, 1959)
Year:	GLP: no
Test substance:	other TS: Commercial product
Method:	0.1 ml of a 20% acetone solution of the sample (equivalent to 20 mg of the test material) was applied to a ¾ x 7/8 inch piece of filter paper. After the acetone had evaporated, the filter paper was place on the skin of 15 human subjects. Nine patch applications were made to the same location on the upper arm over a period of two weeks. A challenge patch was applied to skin area not previously exposed to the test material.
Result:	None of the 15 subjects tested exhibited any evidence of sensitization.

09-NOV-2001 (7)

5.4 Repeated Dose Toxicity

-

5.5 Genetic Toxicity 'in Vitro'

Type:	Ames test
System of testing:	Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100
Concentration:	0.0005, 0/001, 0.0025, 0.005, 0.01, 0.05, 0.1, 0.5 ug/plate
Cytotoxic Conc.:	Without metabolic activation: >0.07 ug/plate; Precipitation conc: 0.59 ug/plate
Metabolic activation:	with and without
Result:	negative
Method:	other: Ames Salmonella/Microsome Plate Test, Protocol 401, Edition 14
Year:	GLP: yes
Test substance:	other TS: Commercial product, purity >95%
Remark:	Examination of mutagenic activity in the presence and absence of liver microsomal preparations was conducted. Solvent control (dimethyl sulfoxide) and specific positive control compounds were assayed concurrently with the test material. The concurrent solvent control data were used as a basis for evaluating results.
Result:	The test material did not exhibit genetic activity in any of the assays conducted and was not mutagenic to the S. typhimurium indicator organism under the test conditions.
Reliability:	(1) valid without restriction GLP Guideline study
Flag:	Critical study for SIDS endpoint

09-NOV-2001 (8)

5.6 Genetic Toxicity 'in Vivo'

-

5.7 Carcinogenicity

-

5.8 Toxicity to Reproduction

-

5.9 Developmental Toxicity/Teratogenicity

-

5.10 Other Relevant Information

-

5.11 Experience with Human Exposure

-

(1) From internal technical bulletin, 1981

(2) Evaluation as part of Certificate of Analysis, by Fine Pharmaceutical Laboratories, Ltd., Hamilton, Ontario, Canada, January 24, 2001

(3) Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.

(4) Unpublished study, "Acute Oral Adminstration of UOP 604 and UOP 688 to Rats", Hill Top Research Institute, Inc. Miamiville, OH, February 13, 1963

(5) Unpublished study, "Acute Dermal Toxicity, Primary Skin Irritation and Acute Eye Irritation Potential of UOP 688", Hill Top Research, Inc., Cincinnati, OH, September 22, 1981

(6) Unpublished study, "Skin Corrosivity Study of UOP 688 in Rabbits (DOT/UN Regulations)", Hazelton Wisconsin, Inc, Madison WI, June 25, 1993.

(7) Unpublished study, "Repeated Insult Patch Test of UOP 688 and 12267", Hill Top Research, Inc., September 20, 1962.

(8) Unpublished study, "Mutagenicity Test on XPA-28-86/UOP 688 in the Ames Salmonella/Micorsomal Reverse Mutation Assay", Hazelton Laboratories America, Inc., Kensington, MD, October 13, 1981.

7.1 End Point Summary
-

7.2 Hazard Summary
-

7.3 Risk Assessment
-

101-72-4
1,4-Benzenediamine, N-(1-methylethyl)-N'-phenyl-

Molecular Weight: **226.32**
Molecular Formula: **C15-H18-N2**

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance: Organic
B. Physical State: Dark purple-brown to dark grey solid
C. Purity: 96-98 % Typical for Commercial Products

1.2 SYNONYMS

Santoflex® IPPD
Santoflex® IP
Flexzone® 3
Vanox® 3C
Vulkanox® 4010NA
Permanax® IPPD
N-Isopropyl-N'-p-phenylenediamine
N-Isopropyl-N'-phenyl-1,4-phenylenediamine
4-(Isopropylamino)diphenylamine
Phenylisopropyl-p-phenylenediamine
IPPD

1.3 IMPURITIES

Diisopropyl PPD isomers (2) 1.0-2%
4-Aminodiphenylamine (CAS# 101-54-2) <1.5%

1.4 ADDITIVES

None

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	75-80°C
Decomposition:	No
Sublimation:	No
Method:	ASTM D-1519/FF83.9-1
GLP:	Yes
Remarks:	Capillary Melt Point determination
Reference:	ASTM D-1519/Flexsys Standard Methods of Analysis, 1983
Reliability:	(1) Valid without restriction

*2.2 BOILING POINT

Value:	>350°C
Pressure:	1013 hPa
Decomposition:	No
Method:	Instrumental – DSC Thermal Stability, 2002
GLP:	Yes
Remarks:	Sample was run from ambient temperature to 350° at 10°/minute Straight baseline with no endotherm after melt, indicating thermal

stability.
Reference: Flexsys Analytical Research Report AP2002.118, 2002
Reliability: (1) Valid without restriction

Value: 161 °C
Pressure: 1.333 hPa
Decomposition: No
Method: No data
GLP: No data
Remarks: Reduced pressure boiling point @ 1mm Hg
Reference: Monsanto Toxicology Profile of Santoflex IP, 1990
Reliability: (4) Unassignable – no details

Value: 341.75 °C
Pressure: 1013 hPa
Method: MPBPWIN v1.40 .
GLP: No
Remarks: Adapted Stein & Brown Method, calculated based on molecular structure and melt point of 75°C, water solubility of 6.7 mg/l and Log P of 3.88
Reference: EPISUITE/MPBPWIN v1.40
Reliability: (2) Valid with restrictions – modelling data

†2.3 DENSITY (relative density)

Type: Density
Value: 1.18
Temperature: 20 °C
Method: FF97.8-1 Density of Solids
GLP: Yes
Remarks: Density of solids by displacement
Reference: FF97.8-1 Flexsys Standard Methods of Analysis, 1997
Reliability: (1) Valid without restriction

***2.4 VAPOUR PRESSURE**

Value: 0.00457 hPa
Temperature: 90 °C
Method: No data
GLP: No data
Remarks: Elevated temperature vapor pressure
Reference: Monsanto Toxicology Profile of Santoflex IP, 1990
Reliability: (4) Unassignable – no details

Value: 0.000093 hPa
Temperature: 25 °C
Method: MPBPWIN v1.40
GLP: No data
Remarks: Modified Grain Method, calculated based on molecular structure and melt point of 75°C, water solubility of 6.7 mg/l and Log P of 3.88. Modelling data suggests that this compound will exist in both the vapor and particulate phases if released to air. Vapor-phase compound will be rapidly degraded by photochemically-

	produced hydroxyl radicals; particulate-phase compound will be removed by wet and dry deposition.
Reference:	EPISUITE/MPBPWIN v1.40
Reliability:	(2) Valid with restrictions – modelling data

***2.5 PARTITION COEFFICIENT $log_{10}P_{ow}$**

Log Pow:	3.88
Temperature:	25°C
Method:	Measured HPLC Method for Pow, 1978
GLP:	No
Remarks:	1% and .01% solutions in 100 ml n-Octanol added to 500 ml water. Shaken for 48 hours, equilibration for several days. Equilibration performed in the dark to preclude photodegradation. Analysis via HPLC to determine Pow; Pow = 7600 +/- 1400
Reference:	Monsanto ES-78-SS-20, 1978
Reliability:	(2) Valid with restrictions – lack of method detail

Log Pow:	3.28
Temperature:	Not applicable
Method:	calculated SRC LogKow (KowWin) Program 1995
GLP:	No
Remarks:	Calculation based on molecular structure and melt point of 75°C and a water solubility of 6.7 mg/l.
Reference:	Meylan, W.M. and. P.H. Howard, 1995 J. Pharm. Sci. 84: 83-92
Reliability:	(2) Valid with restrictions – modelling data

***2.6 WATER SOLUBILITY**

A. Solubility

Value:	7.6 mg/l at pH 7.0
Temperature:	25 °C
Description:	Of very low solubility
Method:	Saturated Solution / Solvent Extraction / GC Analysis
GLP:	Yes
Remarks:	Test substance was added to buffered and pH-adjusted water, stirred for 1 hour while shielded from light. The solution was filtered, extracted with methylene chloride, and dried through sodium sulfite. The methylene chloride was evaporated to near dryness, then acetone was added and evaporated again. This was transferred to a 10 ml volume with acetone and analyzed via GC.
Reference:	Monsanto ABC-32301, Analytical Bio-Chemistry Labs, 1986
Reliability:	(1) Valid without restriction

Value:	15 ppm
Temperature:	25 °C
Description:	Of very low solubility
Method:	Saturated Solution / Solvent Extraction / GC Analysis
GLP:	No data

Remarks:	CH2Cl2 solvent, 100% recovery at 1 ppm. Equilibrated w/out light.
Reference:	Monsanto ES-78-SS-20, Environmental Sciences, 1978
Reliability:	(2) Valid with restrictions – lack of method detail

B. pH Value, pKa Value

pH Value:	Not Applicable
pKa value	5.1 at 25°C
Method:	Estimated
GLP:	No
Remarks:	Value indicates that this compound will exist only slightly in the cation form.
Reference:	HSDB database 101-72-4, SRC, University of Georgia SPARC SPARC On-Line Calculator
Reliability:	(2) Valid with restrictions – modelling data

2.7 FLASH POINT

Value:	150.5°C
Type:	Cleveland Open Cup
Method:	ASTM D 92-96
Reference:	Flexsys America Data
Reliability:	(1) Valid without restrictions

2.11 OXIDISING PROPERTIES

†2.12 OXIDATION: REDUCTION POTENTIAL

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

B. Other data

Results:	Henry's Law Constant = 1.4 x 10(-9) atm-cu m/mole
Remarks:	Fragment Constant Estimation method. Volitilization from moist soil surfaces is not expected to be an important fate process.
Reference:	EPIWIN/HENRYWIN v3.10
Reliability:	(2) Valid with restrictions – modelling data

3. <u>ENVIRONMENTAL FATE AND PATHWAYS</u>

***3.1.1 PHOTODEGRADATION**

Type:	Air
Indirect Photolysis:	
Type of sensitizer:	OH
Concentration of sensitizer:	156000 molecule/m3
Rate constant (radical):	218.3766 E-12 cm^3/molecule-sec
Degradation:	50% after 0.588 hours
Method:	calculated AOP Program v1.90, 2001
GLP:	No

Test substance:	Other: Calculation based on molecular structure and melt point of 75°C, water solubility of 6.7 mg/l and Log P of 3.88
Reference:	EPIWIN/AopWin v1.90
Reliability:	(2) Valid with restrictions – accepted calculation method

***3.1.2 STABILITY IN WATER**

Type:	Abiotic (hydrolysis)
Half life:	Not Determined
Degradation:	99% at pH 7.0 at 25 °C after 24 Hours
Method:	Extraction, ABC Protocol M-8305 (1986)
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 97%
Remarks:	Primary stock solutions of 1.00 mg/l of the test compound were prepared in nanograde acetone. Subsequent dilutions for spiking and gas chromatography standards were also prepared in nanograde acetone. Test samples were extracted with three 75ml portions of methylene chloride. The extracts were dried by passing them through a funnel containing anhydrous sodium sulfate. No test substance detected at seven days. Hydrolysis products identified by GC analysis and confirmed by GS/Mass Spectrometry as 4-hydroxydiphenylamine (18%) and Benzoquinoneimine-n-phenyl (81%). The Benzoquinoneimine-n-phenyl is the oxidized form of 4-hydroxydiphenylamine (CAS# 122-37-2, C12-H11-N-O). The amine portion of the test compound molecule was not isolated, nor was it apparent from the GC-MS spectra. It was postulated that the amine portion might be present in the hydrolysis water layer, indicating that the linkage was cleaved at the aromatic carbon-nitrogen bond.
Reference:	Monsanto ABC-32301, Analytical Bio-Chemistry Labs, 1986
Reliability:	(1) Valid without restriction

***3.2 MONITORING DATA (ENVIRONMENTAL)**

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

***3.3.1 TRANSPORT**

Type:	Volatility
Media:	Water
Method:	Calculation from EPIWIN VP/WS 2001
Results:	Volatilization half-life from model river: 6.117E+005 hours Volatilization half-life from model lake: 6.673E+006 hours Volatilization Constant from water: 1.44E-009 atm-m3/mole
Remarks:	Model river = 1 m deep flowing at 1 m/sec and wind velocity of 3 m/sec. Model lake = 1 m deep flowing at 0.05 m/sec and wind velocity of 0.5 m/sec. Calculation based on molecular structure and melt point of 75°C, water solubility of 6.7 mg/l and Log P of 3.88

Reference: EPISUITE/EPIWIN 2001
Reliability: (2) Valid with restrictions – modelling data

*3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota-sediment-soil-water
Method: Fugacity level III
Results:

	Mass Amount (%)	Half-life (hrs)	Emissions (kg/hr)
Air	0.0155	1.18	1000
Water	21.4	900	1000
Soil	76.3	900	1000
Sediment	2.27	3.6E+003	0

Persistence time estimated at 940 Hours
Remarks: Calculation based on molecular structure and melt point of 75°C, water solubility of 6.7 mg/l and Log P of 3.88
Reference: EPISUITE/EPIWIN v3.10
Reliability: (2) Valid with restrictions – modelling data

*3.5 BIODEGRADATION

Type: aerobic
Inoculum: adapted
Concentration of the chemical: 1002 ug/l related to test substance
Medium: water
Degradation: 50% after 2.5 Hours
90 % after 3.5 Hours
98% after 22 Hours
Results: readily biodegradable
Method: Primary Biodegradation by Natural Water Die-Away Test, Dixon, Hicks and Michael, 1981
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97%.
Remarks: Tests run in Mississippi river water collected on 4/27/81 at the St. Louis waterfront and on purified Milli-Q water. A portion of the river water was sterilized by membrane filtration. A second portion was filtered through glass wool to remove particulates without elimination of the active biomass. The short half-lives in both systems suggest that the compound should not persist in natural aquatic environments.
Reference: Monsanto ES-81-SS-53, MIC Environmental Sciences, 1981
Reliability: (1) Valid without restriction

Type: aerobic
Inoculum: adapted
Concentration of the chemical: No data
Medium: wastewater and activated sludge
Degradation: 90-98% (no time specified)
Results: readily biodegradable
Method: No data
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: not specified

Remarks: The continuous aeration and biodegradation with activated sludge of wastewaters from the manufacture of the test compound removes most of the chemical. The biological oxygen demand decreases by 90-98%. The remaining colored substances are removed by adsorption on activated carbon. Dilution with surface water removes any residual phytotoxic activity.
Reference: Regula et al., Chem. Prum. 33(4), 212-125, 1983
Reliability: (4) Unassignable – data from a secondary literature source

Type: aerobic
Inoculum: adapted
Concentration of the chemical: 30.4 mg/l related to test substance
Medium: water
Degradation: 18.9% of theory CO2 evolution after 32 days
Results: not readily biodegradable
Method: Ultimate Biodegradation by Monsanto Shake Flask Procedure, Gledhill, Appl. Microbiol. 30, 922 (1975)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97%.
Remarks: In the shake flask procedure, 60ml of acclimated bacterial seed is mixed with 440 ml of minimal salts media in a fluted 2-l Erlenmeyer flask. A weighed quantity of the test material is added. The solution is aerated with 70% oxygen in nitrogen. An open reservoir containing 10 ml of 0.2N barium hydroxide is suspended via a glass tube inserted in a rubber stopper. Provisions for removal and addition of the barium hydroxide solution, aeration and sampling are provided. Flasks are agitated on a rotary shaker at 80 rpm, in the dark, and at ambient temperature. Samples are removed at 3, 7, 14, 21, 38 and 35 days for analysis. CO2 values obtained with the control are subtracted from values for the test material. Considering the rapid primary degradation of the test compound in the River Die-Away Test, the failure to obtain significant CO2 evolution suggests formation of more persistent metabolites or degradation products.
Reference: Monsanto ES-78-SS-28, MIC Environmental Sciences, 1978
Reliability: (1) Valid without restriction

3.6 BOD5, COD or BOD5/COD Ratio

3.6 BIOACCUMULATION

Species: Other
BCF: 193.9
Method: BCFWIN v2.14
GLP: No
Remarks: Calculation based on molecular structure and melt point of 75°C, water solubility of 6.7 mg/l and Log P of 3.88
Reference: EPIWIN/BCFWIN v2.14
Reliability: (2) Valid with restrictions – modelling data

Species: Other
BCF: 170 (+/-20)
Method: Neely et al., 1974 (Calculation from measured Log Pow)

GLP:	No
Remarks:	Calculation based on measured Log Pow value of 3.88 Good agreement with BCFWIN model
Reference:	Monsanto ES-78-SS-20, Environmental Sciences, 1978
Reliability:	(2) Valid with restrictions – lack of method detail

4. ECOTOXICITY

*4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type of test:	static Closed system
Species:	Salmo gairdneri (Rainbow Trout)
Exposure period:	96 Hours
Results:	LC_{50} (24h) = 0.62 mg/l LC_{50} (48h) = 0.38 mg/l LC_{50} (96h) = 0.34 mg/l NOEC = 0.18 mg/l LOEC = 0.24 mg/l
Analytical monitoring:	No
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: 97%
Remarks:	The test material, in reagent-grade Acetone, was introduced into 15 liters of diluent water in all-glass vessels. Nominal test concentrations (duplicate) were 0, 0.18, 0.24, 0.32, 0.42, 0.56 and 0.75 mg/l, plus a solvent control. To each test vessel, 10 rainbow trout, standard length 3.7 cm, were then added. The test fish were not fed 48 hours prior to testing, nor during exposure. No aeration was provided during the test, and temperature was maintained at 12°C. Dissolved oxygen ranged from 9.0 mg/l (84% saturation) to 3.4 mg/l (32% saturation) from beginning to end of exposure, respectively. pH values ranged from 7.3 initially, to 6.9 at the end of the test. Observations and mortality counts were made every 24 hours. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50s and the 95% confidence intervals were calculated from the regression equation.
Reference:	Monsanto BN-76-255, EG&G Bionomics, 1977
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

Type of test:	static Closed system
Species:	Lepomis machrochirus (Bluegill Sunfish)
Exposure period:	96 Hours
Results:	LC_{50} (24h) = 0.48 mg/l LC_{50} (48h) = 0.43 mg/l LC_{50} (96h) = 0.43 mg/l NOEC = 0.24 mg/l LOEC = 0.32 mg/l
Analytical monitoring:	No

Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: 97%
Remarks:	The test material, in reagent-grade Acetone, was introduced into 15 liters of diluent water in all-glass vessels. Nominal test concentrations (duplicate) were 0, 0.24, 0.32, 0.42, 0.56 and 0.75 mg/l, plus a solvent control. To each test vessel, 10 bluegill, standard length 2.3 cm, were then added. The test fish were not fed 48 hours prior to testing, nor during exposure. No aeration was provided during the test, and temperature was maintained at 22°C. Dissolved oxygen ranged from 8.8 mg/l (100% saturation) to 0.4 mg/l (5% saturation) from beginning to end of exposure, respectively. pH values ranged from 7.3 initially, to 6.8 at the end of the test. Observations and mortality counts were made every 24 hours. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50s and the 95% confidence intervals were calculated from the regression equation.
Reference:	Monsanto BN-76-255, EG&G Bionomics, 1977
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

Type of test:	flow-through (dynamic) Closed system
Species:	Pimephales promelas (Fathead Minnows)
Exposure period:	14 days
Results:	$LC_{50\ (24h)}$ = 1.80 mg/l $LC_{50\ (192h)}$ = 0.28 mg/l $LC_{50\ (240h)}$ = 0.21 mg/l $LC_{50\ (336h)}$ = 0.09 mg/l
Analytical monitoring:	Yes
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 97%
Remarks:	The test fish, mean standard weight 0.99g and mean standard length 43.0 mm, were obtained from Fattig's Fish Hatchery in Brady, Nebraska. The fish were held in culture tanks on a 16-hour photoperiod and were observed for at least 14 days prior to testing. During the holding, acclimation and test periods, the fish were fed a standard commercial fish food daily in an amount equivalent to 3% of body weight. As a quality check, the fish were challenged with a reference compound, Antimycin A, prior to the test. The observed 96hr LC50 and 95% confidence limits indicated that the fish were in good condition. A proportional diluter system was used for the intermittent introduction of the test article, in nanograde acetone, and diluent water, into the test aquaria. Aerated well water, hardness 250 mg/l and alkalinity 360 mg/l, pH 7.7 and dissolved oxygen 9.3 mg/l, was delivered to the glass aquaria at the rate of 300ml/minute, an amount which provided replacement of the 30 liter volume at least 14 times in each 24-hour period. The temperature in the test aquaria was held

at 22°C. Water quality parameters of temperature, dissolved oxygen (100-60%), pH (7.7-7.9) and ammonia (0.20-1.8) were monitored throughout the test and remained within acceptable limits. Thirty test fish/aquaria were exposed to concentrations of 0, 0.066, 0.12, 0.23, 0.45 or 1.0 mg/l of the test article for the 14-day test period. Observations for mortality and abnormal behavior were performed once/day. Concentrations of the test article were determined by IR spectroscopy using a calibration curve determined from known concentrations with the addition of Rhodamine B dye. The concentrations were further confirmed by gas chromatography. The statistical methods described by Litchfield and Wilcoxon were used to determine the LC50 values and the 95% confidence limits. From the acute toxicity curves using both the nominal and mean measured water concentrations, it was determined that the lethal threshold had not been reached after 14 days. The results also indicated that the test article appeared to have cumulative toxicity.

Reference: Monsanto AB78-120B, Analytical Bio-Chemistry Labs, 1979

Reliability: (1) Valid without restriction

Type of test: static
Closed system

Species: Paratanytarsus parthenogenetica (Midge)

Exposure period: 48 Hours

Results: LC_{50} (24h) = 29 mg/l
LC_{50} (48h) = 23 mg/l
NOEC = Not Observed
LOEC = 10 mg/l (lowest concentration tested)

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975) and Gettings and Adams, Method for Conducting Acute Toxicity Tests with Midge 1980

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 97%

Remarks: Test midge for this study were cultured at the ABC facilities. The adult midge were fed a suspension of trout chow and alfalfa daily until 24 hours prior to testing. The test was carried out using 3rd and 4th instar larvae, 8-10 days old. The static bioassay was conducted in 250 ml glass beakers containing 200 ml of ABC well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.2 mg/l, hardness (CaCO3) of 255 ppm and pH 7.8. The test vessels were kept in a water bath at 20°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range finding experiment preceded the definitive bioassay. Nanograde Acetone was used to prepare the test solutions of 10, 18, 32, 56, 100 or 180 mg/l, and as the solvent control. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen content ranged from 8.9 to 7.2 mg/l and pH ranged from 7.9 to 8.5 during the testing. Water quality parameters of temperature, dissolved oxygen content and pH were measured at the termination of the test and were within acceptable limits. The LC50 values were calculated via a computerized program

performing the following statistical tests: binomial, moving average and probit tests.

Reference: Monsanto 9AB981013, Analytical Bio-Chemistry Labs, 1981

Reliability: (1) Valid without restriction

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test: static
Closed system

Species: Daphnia magna

Exposure period: 48 Hours

Results: EC_{50} (24h) = 2.8 mg/l
EC_{50} (48h) = 1.1 mg/l
NOEC = 0.56 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 97%

Remarks: The Daphnia magna used in the test were cultured at the ABC facilities. Adult Daphnia were fed an algae and trout chow mixture daily until 24 hours prior to testing. The bioassay was conducted in 500 ml glass beakers containing 250 ml of ABC well water. During the test, dissolved oxygen concentration ranged from 8.7-8.6 mg/l, pH range was 7.7-8.3, hardness (CaCO3) was 255 mg/l, and alkalinity was >250 mg/l. Vessels were kept in a water bath at 19°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range-finding experiment was carried out to determine the exposure concentrations for the definitive test. Acetone was used as the solvent for the test solutions, and the experiment included both a control and a solvent control (0.01ml). Concentrations (in duplicate) of the test substance were 0, 0.56, 1.0, 1.8 or 3.2 mg/ml. Ten daphnia, first instar less than 18 hours old, were placed in each test chamber. Daphnia in all concentrations were observed once every 24 hours for mortality and abnormal effects. Water quality measurements were monitored throughout the testing and were considered adequate and equivalent to those measurements in the control chamber. Statistical analysis of the concentration vs. effect data was calculated employing the techniques of Litchfield and Wilcoxon (1949).

Reference: Monsanto AB-78-120, Analytical Bio-Chemistry Labs, 1978

Reliability: (1) Valid without restriction

*4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae

Species: Selenastrum capricornutum (Freshwater alga)

Endpoint: Biomass and Growth rate

Exposure period: 96 Hours

Results: EC_{50} (96h) = 0.4 ppm for a chlorophyll, 0.5 ppm for cell numbers
NOEC = <0.1 ppm
LOEC = Not Determined

Analytical monitoring:	No
Method:	US EPA Algal Test Procedure: Bottle Test, 1971 Closed system
GLP:	No data
Results:	The test algae were obtained from the US EPA Environmental Research Laboratory in Corvallis, Oregon. Beginning cell numbers in the test flasks were 2.0 x 10(4) cells/ml. Cultures were incubated at 24°C under approximately 4,000 lux illumination. Triplicate cultures were employed for each of the test concentrations and the control. Test containers were 125ml flasks containing 50ml of test medium. Concentrations for the definitive test were based on the results of a 96-hr range-finding study. These concentrations were 0, 0.1, 0.3, 0.6 1.0 and 3.0 mg/l, plus a solvent control (acetone). The measured pH values ranged from 7.6 to 8.1 during the course of the testing. There were no other water quality measurements besides temperature reported in this study. Statistical analysis involved converting each test concentration to a logarithm, and the corresponding percentage decrease of in vivo chlorophyll a or cell numbers was converted to a probit (Finny, 1971). The EC50s and 95% confidence limits were then calculated by linear regression. The toxicity of the test substance to algae was similar throughout the 96 hours of exposure. There was no significant difference between growth of the control and solvent control cultures after 96 hours of exposure by either measured parameter.
Test substance:	As prescribed by 1.1-1.4, purity: 97%
Remarks:	Both a chlorophyll and cell numbers measured to confirm results.
Reference:	Monsanto BN-78-1384325, EG&G Bionomics, 1978
Reliability:	(2) Valid with restrictions – no GLP statement

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Type:	LD_{50}
Species/strain:	Rats, Sprague-Dawley Albino
Value:	900 mg/kg bw
Sex:	Male and female
# of Animals:	20
Vehicle:	Corn Oil
Doses:	631, 749, 1000 or 1260 mg/kg bw
Method:	Single Oral Dose, Younger Laboratories Protocol, 1973
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: 97%
Remarks:	Four groups of male and female rats (5/sex/dose level) were fed a single oral dose of the test article as a 20% suspension in corn oil warmed to 115°F via oral gavage. Male rats had initial average body weights of 210-230 grams: females had initial average body weights of 210-235 grams. Clinical signs of toxicity included reduced activity and appetite for 3-5 days for survivors, and increasing weakness, collapse and death for decedents in 1-5 days, with most deaths occurring within 2 days. Gross autopsy findings

on decedents were lung hyperemia, slight liver discoloration, and acute gastrointestinal inflammation. Survivors were sacrificed after fourteen days. All viscera of survivors appeared normal. 95% confidence limits 850-950 mg/kg.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
631	0/3	0/2	0/5
749	0/2	2/3	2/5
1000	2/3	2/2	4/5
1260	2/2	3/3	5/5

Reference: Monsanto Y-73-287, Younger Laboratories, 1974
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.1.2 ACUTE INHALATION TOXICITY

Type: LC_0
Species/strain: Mice
Value: >90 mg/m3
Exposure time: 4 hours daily
Sex: No data
# of Animals: No data
Vehicle: None – fine dust
Doses: 10-90 mg/m3
Method: No data
Test substance: As prescribed by 1.1-1.4, purity: No data
Remarks: The inhalation of the test compound at concentrations between 10 and 90 mg/m3 by mice for 4 hours/day did not cause any mortalities. Irritation of the bronchial tubes and minor damage to the lungs were described.
Reference: Mel'nikova, L.V., cited in Chem. Abstracts, 1967
Reliability: (4) Unassignable – data from a secondary literature source

5.1.3 ACUTE DERMAL TOXICITY

Type: LD_{50}
Species/strain: Rabbits, New Zealand Albino
Exposure time: 24 Hours
Sex: Male and female
# of Animals: 3
Vehicle: Corn Oil
Value: >7940 mg/kg bw
Method: Single Dermal Dose, Younger Laboratories Protocol, 1973
Test substance: As prescribed by 1.1-1.4, purity: 97%
Remarks: The test article, as a 40% suspension in corn oil, was applied to the shaved skin of two male and one female rabbits at dose levels of 5010 or 7940 mg/kg bw. Males in this study weighed 2.1 and 2.2 kg initially, and the female weighed 2.0 kg. The test material was held in place by means of an occlusive wrap of latex rubber and secured by bandaging and elastic tape. The occlusive wrap was removed after 24 hours and the excess material was wiped from the test animal. Clinical observations were made three times during the first eight hours after dosing, and twice daily thereafter

until sacrifice. Clinical signs of toxicity noted were reduced appetite and activity for three to five days. All test animals survived. Survivors were sacrificed after 14 days. All viscera in survivors appeared normal.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
6310	0/1	---	0/1
7940	0/1	0/1	0/2

Reference: Monsanto Y-73-287, Younger Laboratories, 1974
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.2.1 SKIN IRRITATION/CORROSION

Species/Strain: Rabbits, New Zealand Albino
Sex: Male and female
# of Animals: 6
Vehicle: Water
Value: 0.0/0.0
Results: Not Irritating
Classification: Non-Irritating
Exposure Time: 24 Hours
Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 97%
Remarks: 0.5 ml of the test substance as a finely ground powder moistened with water was applied to the shaved dorsal areas of six albino rabbits. The test material was applied to the skin under 1" square gauze patches and held in contact with the skin by means of an occlusive wrap of latex rubber secured by bandaging and elastic tape. The occlusive wrap and gauze patches were removed after 24 hours. Dermal irritation was scored by the Draize Method, and results were recorded 24, 48, 72 and 168 hours after topical application. The Primary Irritation Index was calculated by averaging the mean scores at 24 and 72 hours. All animals scored zero (0) at every observation time.
Reference: Monsanto Y-73-287, Younger Laboratories, 1974
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.2.2 EYE IRRITATION/CORROSION

Species/strain: Rabbits, New Zealand Albino
Sex: Male and female
# of Animals: 6
Vehicle: None
Value: 1.3/110.0
Results: Slightly irritating
Classification: Non-irritating
Exposure Time: 24 Hours
Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP: No data
Test substance: As prescribed in 1.1-1.4, purity: 97%

Remarks: 100.0 mg of the undiluted test substance as a finely ground powder was applied to one eye of six albino rabbits. The other eye was not treated and served as a control. The cornea, iris and conjuntivae were examined immediately after treatment, and then at intervals of 10 minutes, 1 hour, and at 24, 48, 72 and 168 hours. The Draize Method was used for scoring eye irritation. Immediate findings: slight discomfort.
At 10 minutes: Slight erythema and discharge
At 1 hour: slight erythema and discharge
At 24 hours: slight erythema and discharge
At 48 hours: all animals scored "0"
At 72 hours: all animals scored "0"
The average Draize score for 24, 48 and 72 hours was calculated for each animal and then averaged over the six animals. The average Draize score was 1.3 on a scale from 0-110.

Reference: Monsanto Y-73-287, Younger Laboratories, 1974

Reliability: (2) Valid with restrictions – age of study, lack of method detail

***5.3 SENSITIZATION**

Type: Repeated Insult Patch Test
Species/strain: Human
Results: Sensitizing
Classification: Sensitizing
Method: Shelanski and Shelanski, 1976
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >96%
Remarks: Santoflex IP, 50% w/v in Dimethylphthalate, was applied to the upper arm of 50 human volunteers using a linteen disk moistened with the test material. The patch was kept in place for 24 hours before removal and grading of gross skin changes on a scale of 0-4. After a 24-hour rest period, the test material was reapplied. This cycle was repeated every Monday, Wednesday and Friday, with a 48-hour rest period over weekends. After the 15th application, the volunteers rested two weeks before the challenge application.

Application #1:	Score 0/50
Applications #2-15:	Score 10/50
Challenge:	Score 11/50

Under the test conditions, 11/50 or 22% of the volunteers showed sensitization responses. Those 11 persons were also subjected to a supplementary challenge using Santoflex 13 (6PPD). No subject showed any indication of cross-sensitization from one PPD rubber chemical material to another.

Reference: Monsanto SH-76-7, Product Investigations, Inc., 1976

Reliability: (2) Valid with restrictions – age of study, lack of method detail

Type: Modified Draize Skin Sensitization
Species/strain: Human
Results: Sensitizing
Classification: Sensitizing
Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >96%

Remarks: The study was performed over a 6-week period on 82 human volunteers using Santoflex IP, 1%, in petrolatum. During the first three weeks, patches moistened with the test material were applied to the arms at the same site at the rate of three times/week. Following a rest period, a challenge application was made to a different site. Results for irritation and sensitization were scored on a scale of 0-4. 12 of 82 test subjects were deemed to be sensitized, for a rate of 14.6%
Reference: Monsanto MA-78-92, Howard Maibach, M.D.,1978
Reliability: (2) Valid with restrictions – age of study, lack of method detail

Type: Open Epicutaneous Test
Species/strain: Guinea Pig
Results: Sensitizing
Classification: Sensitizing
Method: No data
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: No data
Remarks: None
Reference: Barlogova, S. et al., 1985
Reliability: (4) Unassignable – data from a secondary literature source

Type: Maximization Test
Species/strain: Guinea Pig
Results: Sensitizing
Classification: Sensitizing
Method: Guinea Pig Maximization Test
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: No data
Remarks: None
Reference: Herve-Bazin, B. et al., 1977
Reliability: (4) Unassignable – data from a secondary literature source

*5.4 REPEATED DOSE TOXICITY

Species/strain: Rats, Sprague-Dawley Albino
Sex: Male/Female
# of Animals: 50 (5/sex/group)
Route of Administration: Oral feed
Exposure period: 28 days
Frequency of treatment: Daily
Post exposure observation period: None
Dose: 0, 500, 1000, 1750 or 2500 ppm
Control group: Yes
Concurrent vehicle
NOEL: 500 ppm
LOEL: 1000 ppm
Results: In a 30-day range-finding study that preceeded a 90-day study, the test substance was administered orally, via dietary admixture, to groups of male and female rats (5/sex/group). Control animals received the standard laboratory diet. Concentration and stability of the test article in the feed admixture was determined/confirmed via gas chromatography. Physical observations, body weight and food consumption measurements were performed on all animals

pretest and at selected intervals during the study. Hematology and chemistry determinations were performed on all animals at study termination. There were no mortalities during the course of the study. After four weeks of treatment, all animals were sacrificed, selected organs were weighed, and organ/body weight ratios were calculated. Complete postmortem examinations were conducted on all animals. Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure. Bartlett's test was performed to determine if there was equal variance. Dunnett's test was used to determine which means were significantly different from the control. Differences from control in body weight gain, hematological effects, elevations in total serum protein and increased liver and spleen weights for both males and females were noted in animals dosed at 1000 ppm and above. There were no significant differences in findings between control groups animals and those dosed at 500 ppm that were attributed to the test article.

Method: OECD Guidelines for Testing of Chemicals, Section 412, 1981
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97.2%
Reference: Monsanto BD-88-74, Bio/dynamics Inc. 1988
Reliability: (1) Valid without restriction

Species/strain: Rats
Sex: No data
# of Animals: No data
Route of Administration: Inhalation
Exposure period: 15 days
Frequency of treatment: 2 hours/day
Post exposure observation period: No data
Dose: 300-400 mg/m3
Control group: Yes
No treatment
NOEL: No data
LOEL: No data
Results: No differences in body weight gain between treated rats and untreated control animals. No differences were noted in the weights of the kidneys or hearts. No morphological changes were noted in any of the organs examined. The functional state of the nervous system of some rats changed. Liver malfunctions and decreased weight of the liver were noted.
Method: No data
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: commercial grade
Reference: Vorob'eva, et al., Soviet Rubber Technology, 1963
Reliability: (4) Not assignable – data from a secondary literature source

Species/strain: Rats, Sprague-Dawley Albino
Sex: Male/Female
# of Animals: 80 (10/sex/dose)
Route of Administration: Oral/Dietary

Exposure period: 90 Days
Frequency of treatment: Daily
Post exposure observation period:
Dose: 0, 180, 360 or 720 ppm
Control group: Yes
Concurrent vehicle
NOEL: 180 ppm for males, could not be determined for females
LOEL: 360 ppm for males, 180 ppm for females
Results: The test substance was administered orally, via dietary admixture, to groups of male and female rats (10/sex/group). Control animals received the standard laboratory diet. Concentration and stability of the test article in the feed admixture was determined/confirmed via gas chromatography. Test rats were 47 days old at initiation of treatment. Mean weight of males was 197 grams (range 182-213 grams); mean weight of females was 154 grams (range 143-167 grams). Physical observations, body weight and food consumption measurements were performed on all animals pretest and at selected intervals during the study. Hematology and chemistry determinations were performed on all animals at Months 1.5 and 3. One high-dose and one mid-dose female were found dead on test day 93 following collection of terminal blood samples. The cause of death was attributed to the stress of bleeding and not to the administration of the test article. There were no other mortalities during the course of the study. After three months of treatment, all animals were sacrificed, selected organs were weighed, and organ/body and organ/brain weight ratios were calculated. Complete postmortem examinations were conducted on all animals. Histopathological evaluation of selected tissues was performed on all control and high-dose animals. The lungs, spleen, liver and kidneys were examined microscopically for all animals in all groups. Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure. Bartlett's test was performed to determine if had equal variance. Dunnett's test was used to determine which means were significantly different from the control. Mean body weights and mean body weight gains were slightly reduced (2-4%) in males at 750 ppm. Treatment-related findings were observed in several hematology parameters in the males and/or females at dose levels of 360 and 720 ppm. Parameters affected included reduced hemoglobin concentrations and hematocrit values at Week 6, reduced hemoglobin concentration in 720 ppm females at Week 13, elevated platelet counts in males at Week 6, and reduced mean erythrocyte counts in females at Week 6 and in high-dose females only at Week 13. The NOEL for hematology data was set at 180 ppm for both sexes. Differences in clinical chemistry parameters were noted in all mid- to high-dose animals. Mean liver weights, liver-to-body-weight and liver-to-brain-weight ratios were increased in 360 and 720 males, and in all treated females. There were no treatment-related findings noted in mortality, physical observations, opthalmology, food consumption or gross or microscopic pathology in any dose/sex group.
Method: OECD Guidelines for Testing of Chemicals, Section 453, 1981 and US EPA TSCA Section 4(a) Test Rules, 1982

GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97.2%
Reference: Monsanto BD-88-389, Bio/dynamics, Inc. 1990
Reliability: (1) Valid without restriction

*5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

Type: Bacterial Reverse Mutation - Ames
System of testing: TA-98, TA-100, TA-1535, TA-1537, TA-1538
Concentration: 0.1, 1, 10, 100 and 500 micrograms/plate
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 500 ug/plate
Without metabolic activation: 500 ug/plate
Precipitation conc: Not determined
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Ames Plate Test (Overlay method) 1975
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97%
Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were 10 ug/plate Methylnitrosoguanidine (MNNG), 100 ug/plate 2-nitrofluorene (NF) or 10 ug/plate Quinacrine mustard (QM). Positive controls used for the activation assays were 100 ug/plate 2-anthramine (ANTH), 100 ug/plate 2-Acetylaminofluorene (AAF) or 100 ug/plate 8-Aminoquinoline (AMQ). Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Positive control treatments produced the expected large increases in the frequency of histidine revertants. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.
Reference: Monsanto BIO-76-226, Litton Bionetics, 1976
Reliability: (1) Valid without restriction

Type: Bacterial Reverse Mutation - Ames
System of testing: TA-98, TA-100, TA-1535, TA-1537
Concentration: 0.2, 0.8, 4, 20, 60 and 200 micrograms/plate
Metabolic activation: With and without
Results:

Cytotoxicity conc: With metabolic activation: 200 ug/plate
Without metabolic activation: 200 ug/plate
Precipitation conc: Insoluble at 1 mg/plate and above
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Ames Plate Test (Overlay method) 1975
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 92-99%
Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. It was purchased from Litton Bionetics, Inc. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Three replicate plates were prepared for each strain/S9/dose level. Concurrent positive and negative controls were conducted for plate incorporation tests to demonstrate strain sensitivity and metabolic activation system capability. Statistical analysis was performed on plate incorporation assay results after transforming revertant/plate values as log 10 (revertants/plate). Analysis included Bartlett's test for homogeneity of variance and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubb's test was performed to determine if outliers were present. Statistical significance of dose response was evaluated by regression analysis. A toxicity screen was conducted using test strain TA100, with and without S9 mix. The test sample was toxic at levels of 200 ug/plate and above. In the definitive test, the test compound was not mutagenic towards any tester strain, with or without metabolic activation.
Reference: Monsanto ML-85-243, Environmental Health Labs, 1986
Reliability: (1) Valid without restriction

Type: Mitotic Recombination Assay
System of testing: Saccharomyces cerevisiae, D4
Concentration: 0.1, 1, 10, 100 and 500 micrograms/plate
Metabolic activation: With and Without
Results:
Cytotoxicity conc: With metabolic activation:
Without metabolic activation.
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Ames Mutagenicity Plate Test (Overlay Method) 1975
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97%
Remarks: The test compound was evaluated for genetic activity in assays with and without the addition of mammalian metabolic

activation preparations. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. The chemical used as the positive control for the non-activation assay was methylnitrosoguanidine (MNNG) at 10 ug/plate. Positive control chemical used for the activation assay was DMNA at 100 micromoles/plate. Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Statistical analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present.The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: Monsanto BIO-76-226, Litton Bionetics, 1976
Reliability: (1) Valid without restriction

C. NON-BACTERIAL IN VITRO TEST

Type: Mammalian Cell Gene Forward Mutation Assay
System of testing: L5178Y Mouse Lymphoma cells
Concentration: 0.156, 0.313, 0.625, 1.250, 2.500 (without activation)
0.625, 1.250, 2.500, 5.000 and 10.000 (with activation)
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 10.0 ug/ml
Without metabolic activation: 2.5 ug/ml
Precipitation conc: >1 mg/ml
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Clive and Spector, Mutation Research 31:17-29 (1975)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97%
Remarks: The test article was evaluated for specific locus forward mutation in the L5178Y Thymidine Kinase (TK) mouse lymphoma cell assay. The test compound was soluble in DMSO at a concentration of 1 mg/ml. It was tested in the mutation assay at applied doses ranging from 0.0195 to 10 ug/ml in duplicate for both the non-activation and activation tests. This dose range was chosen on on the basis of a preliminary cytotoxicity test which indicated that doses higher than 2.5 ug/ml were highly toxic without activation. In the mutation assay, doses higher than 2.5 ug/ml killed all of the cells within 24 hours of treatment. Less toxicity was observed with activation. Dose levels chosen for completion of the assay were within the range of cytotoxicities where any mutant activity is normally observed. Stock solutions were prepared in DMSO. DMSO (1%) was used as the negative control. EMS (0.5 ul/ml) was used as the positive control without activation and DMN

(0.3 ug/ml) was used as the positive control with activation. No genetic effects were attributed to the presence of the solvent. The reference mutagens EMS and DMN induced mutation frequencies within the expected range.

Non-Activation Results

	Conc.	Mutant clones	Viable clones	Mutant frequency x10(-6)
Solvent Control	-----	36.0	278.0	12.9
Negative Control	-----	19.0	307.0	6.2
EMS	0.5 µl/ml	532.0	76.0	700.0
Test Cpd	0.056	11.0	360.0	3.1
	0.313	32.0	274.0	11.7
	0.625	42.0	382.0	11.0
	1.250	19.0	117.0	10.7
	2.500	79.0	329.0	24.0

Activation with S-9 Results

	Conc.	Mutant clones	Viable clones	Mutant frequency x10(-6)
Solvent Control	-----	46.0	265.0	17.4
Negative Control	-----	52.0	242.0	21.5
DMN	0.3 ug/ml	178.0	112.0	158.9
Test Cpd.	0.625	42.0	318.0	13.2
	1.250	30.0	265.0	11.3
	2.500	37.0	315.0	11.7
	5.000	39.0	299.0	13.0
	10.000	60.0	246.0	24.4

Reference: Monsanto BIO-78-224 Litton Bionetics, 1978
Reliability: (1) valid without restriction

Type: *In vitro* Unscheduled DNA Synthesis (UDS)
System of testing: Primary rat hepatocyte cultures (Fischer-344 strain)
Concentration: 0.01, 0.05, 0.1, 0.5, 1, 3, 5, 10, 50, 100, 1000 ug/ml
Metabolic activation: With and without
Results:
Cytotoxicity conc: Preliminary Assay: 5 ug/ml
Replicate Assay: 3 ug/ml
Precipitation conc: Separation/sticking to sides of tube noted at 100 ug/ml and above
Genotoxic effects: Negative
Method: Williams, G.M., Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures, Cancer Research 37, pp. 1845-1851 (1977)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 92-97%
Remarks: Acetone (1%) used as solvent and diluent. Primary rat liver cell cultures derived from the livers of two adult male rats weighing 313 and 262 grams (21 and 12 weeks old) were used for the preliminary and replicate experiments, respectively. Three controls were incorporated into each UDS assay: a positive control, a negative (solvent) control, and an untreated medium control. The positive control was 2-Acetylaminofluorene (2-AAF), the solvent control was acetone in the preliminary assay and in the replicate assay. The percentage of cells in repair was calculated as the percentage of cells with at least 5 net grains/nucleus. 150 cells were scored for each concentration reported for each experiment. All collection of data and pooling of

slides were done via programs in the VAX 11/782 computer. Cytoxicity was observed at 5, 10, 50, 100, 500 and 1000 ug/ml in the preliminary experiment, and at 3, 5 and 10 ug/ml in the replicate experiment. A separation of test compound sticking to the sides of the tubes was evident at 100 ug/ml and above in the preliminary experiment. UDS was measured at concentrations of the test compound between 0.05 and 1.0 ug/ml in the preliminary experiment, and between 0.01 and 1.0 ug/ml in the replicate experiment. The net grain counts were negative at each concentration of the test compound, in the solvent control and in the medium control, in contrast to the strong positive response produced by the positive control 2-AAF in both experiments (52.9 and 53.4 net grains/nucleus). These results indicate that the test compound is not a genotoxic agent under the conditions of the *in vitro* rat hepatocyte DNA repair assay.

Treatment	Conc.	NG	SE	Median	%IR
Control/medium	---	- 13.0	0.4	-12.5	1
Control/solvent	1%	- 9.0	1.3	- 8.3	2
2-AAF ug/ml	3	53.4	3.1	52.0	99
Test Cpd. ug/ml	0.01	- 8.9	1.7	- 7.3	1
	0.05	- 11.8	2.6	- 11.4	0
	0.10	- 7.4	4.4	- 5.2	5
	0.50	- 10.4	2.2	- 10.4	2
	1.00	- 9.8	1.1	- 9.4	1
	3.00	----------TOXIC----------			

Reference: Monsanto SR-85-251, SRI International, 1986
Reliability: (1) Valid without restriction

Type: CHO/HGPRT Forward Gene Mutation Assay
System of testing: CHO Cells, clone K1-BH4
Concentration: 2, 5, 10, 15 and 30 ug/ml
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 30 ug/ml
Without metabolic activation: 10 ug/ml
Precipitation conc: Not Determined
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: CHO/HGPRT Mutation Assay (1979) Hsie, et.al.
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 92-99%
Remarks: The mutagenic potential of the test substance was evaluated in CHO cells for ability to induce forward mutation at the HGPRT gene locus. A range-finding cytotoxicity study preceded a dose-response mutagenicity experiment using different levels of Arochlor1254 rat liver homogenate (S9) concentrations, followed by a confirmatory dose-response mutagenicity experiment. The compound was tested at S9 concentrations up to a cytotoxic dose of 30 ug/ml. Solutions of the test compound were prepared using DMSO as the solvent on the day of treatment. Positive controls used were benzo(a)pyrene and ethyl methane sulfonate for the

activation and non-activation assays, respectively. The subclone K1BH4 of CHO cells was obtained from Dr. Hsie of Oak Ridge National Laboratories. CHO cells were plated the day before treatment. Statistical analysis was according to the methos of Snee and Irr (1981) designed specifically for the CHO/GHPRT mutation assay. Student's t-test was used to compare treatment data to control data. The Snee and Irr analysis also allowed the determination of dose-response relationship as linear, quadratic, or higher order. A computer program obtained from Joe Irr was used. No statistically significant mutagenicity was observed in the two separate experiments. The positive controls yielded the expected positive responses in mutagenicity, indicating the adequacy of the experimental conditions. Therefore, the test substance was not considered to be mutagenic in CHO cells under the experimental conditions.

Reference: Monsanto ML-85-221, Environmental Health Labs, 1986
Reliability: (1) Valid without restriction

* 5.6 GENETIC TOXICITY IN VIVO

Type: Mammalian Bone Marrow Chromosomal Aberration (SCE)
Species/strain: Mouse
Sex: Male
Route of Administration: Intraperitoneal
Exposure period: Once – single ip dose
Doses: 1, 5, 10, 30, 60 or 120 mg/kg
Results:
Effect on mitotic index or P/N ratio: Negative
Genotoxic effects: Negative
Method: No data
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: No data
Remarks: No increased sister chromatid exchange frequency in mouse bone marrow cells in any dose group.
Reference: Gorecka-Turska, D. et al., 1983
Reliability: (4) Unassignable – data from a secondary literature source

Type: Mammalian Bone Marrow Chromosomal Aberration (SCE)
Species/strain: Mouse
Sex: Male
Route of Administration: Intraperitoneal
Exposure period: Once – single ip dose
Doses: 1-500 mg/kg bw
Results:
Effect on mitotic index or P/N ratio: Negative
Genotoxic effects: Negative
Method: No data
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: No data
Remarks: No statistically significant increase in sister chromatid exchange frequency in mouse bone marrow cells in any dose group.

Reference: Vasilyeva, L.A. et al., 1985
Reliability: (4) Unassignable – data from a secondary literature source

*5.8 TOXICITY TO REPRODUCTION

Type: Other: Reproductive Toxicity Screening
Species/strain: Mice, CD-1
Sex: Female
Route of Administration: Oral gavage
Exposure period: Day 7-14 of gestation
Frequency of treatment: Daily
Post exposure observation period: No data
Duration of the test: No data
Doses: 10 ml/kg for eight days
Control group: Yes
Concurrent vehicle
Results: Screening tests of priority chemicals for possible reproductive hazards were conducted on fifteen compounds in a NIOSH and Centers for Disease Control (CDC) study. Each compound was administered orally to female CD1 mice at 10 ml/kg/day for eight consecutive days. Minimum effective doses (MED) were calculated. The MED was defined as the highest dose that caused a small number of deaths or a significant weight loss. The MED of the test compound was administered on days 7-14 of gestation. Clinical observations were made and necropsies conducted. Mean body weights were obtained daily. Litter size, number of live pups, body weight and body weight changes were recorded and statistically analyzed. The results indicated that the test compound would be a candidate for more detailed reproductive toxicity testing.
Method: No data
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity "commercial"
Remarks: Chemicals tested included phthalate esters, aromatic amines and organophosphates.
Reference: DCN-121196, NIOSH/CDC, 1983
Reliability: (4) Unassignable – data from a secondary literature source

*5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY

Species/strain: Rats, Sprague-Dawley CD
Sex: Female
Route of Administration: Oral gavage
Duration of the test: 20 days from mating to C-section
Exposure period: Day 6-15 of gestation
Frequency of treatment: Daily, as a single oral dose at a volume of 5 ml/kg
Doses: 0, 10, 50 or 100 mg/kg bw
Control group: Yes
Concurrent vehicle
NOEL Maternal Toxicity: 50 mg/kg
NOEL teratogenicity : 100 mg/kg

Results: In a preliminary oral gavage teratology study, forty-three virgin female rats, aged 9-12 weeks at arrival to the test facility, were acclimatized to laboratory conditions for at least 14 days prior to mating. Females were housed with males on a 2 female: 1 male basis. The mating period for this study lasted 4 days. Females showing evidence of mating were separated from the male and designated Day 0 of gestation. The test substance was administered to groups of 24 pregnant rats during the period of embryo organogenesis. The vehicle was Polyethylene Glycol 400, and dose levels were 0, 10, 50 or 100 mg/kg bw. Individual clinical observations, body weight and food consumption were recorded during the study. The animals were sacrificed on Day 20 of gestation, examined macroscopically, and the uterine contents examined. The number of corpora lutea, implantation number, position and type, fetal weights, fetal sex and external appearance were recorded. All live fetuses were preserved, processed and subsequently examined for skeletal or visceral anomalies. Statistical evaluation was performed by the following parameters: Food consumption – one way analysis of variance, followed by pairwise analysis of group values by Student's t-test. Skeletal findings – Chi-square test or Fisher's Exact test for small sample sizes.

Maternal general toxicity: At 100 mg/kg there were signs of post-dosing salivation and lethargy in 5/8 animals, with ptosis noted in one animal. There was also a slight reduction in group mean food consumption over the period of Day 6-Day 9 of gestation. All animals survived to sacrifice. There were no treatment-related macroscopic findings at necropsy for any dose level.

Pregnancy/litter data: There were no treatment-related effects on uterine/implantation at any dose level.

Foetal data: There were no treatment-related effects on fetal parameters.

Reference: Monsanto SP-93-46, SafePharm Laboratories 1994

Reliability: (1) Valid without restriction

Species/strain: Rats, Sprague-Dawley CD

Sex: Female

Route of Administration: Oral gavage

Duration of the test: 20 days from mating to C-section

Exposure period: Day 6-15 of gestation

Frequency of treatment: Daily, as a single oral dose at a volume of 5 ml/kg

Doses: 0, 12.5, 62.5 and 125 mg/kg bw

Control group: Yes
Concurrent vehicle

NOEL Maternal Toxicity: 62.5 mg/kg

NOEL teratogenicity : 62.5 mg/kg

Results: One hundred and thirteen virgin female rats, aged 9-12 weeks at arrival to the test facility, were acclimatized to laboratory conditions for at least 21 days prior to mating. Females were housed with males on a 2 female: 1 male basis. The mating period for this study lasted 12 days. Females showing evidence of mating were separated from the male and designated Day 0 of gestation. The test substance was administered to groups of 24 pregnant rats

during the period of embryo organogenesis. The vehicle was Polyethylene Glycol 400, and dose levels were 0, 12.5, 62.5 or 125 mg/kg bw. Individual clinical observations, body weight and food consumption were recorded during the study. The animals were sacrificed on Day 20 of gestation, examined macroscopically, and the uterine contents examined. The number of corpora lutea, implantation number, position and type, fetal weights, fetal sex and external appearance were recorded. All live fetuses were preserved, processed and subsequently examined for skeletal or visceral anomalies. Statistical evaluation was performed by the following parameters: Food consumption – one way analysis of variance, followed by pairwise analysis of group values by Student's t-test. Skeletal findings – Chi-square test or Fisher's Exact test for small sample sizes.

Maternal general toxicity: High-dose rats exhibited slight maternal toxicity as evidenced by a reduction in food intake, pre-dosing salivation and soft, dark feces. There were no effects on body weight. All animals survived to sacrifice. There were no treatment-related macroscopic findings at necropsy for any dose level.

Pregnancy/litter data: There were no treatment-related effects on uterine/implantation.

Foetal data: At 125 mg/kg there were statistically significant effects on the incidence of skeletal findings. Effects included an increased incidence of irregularly and incompletely ossified cranial and facial bones, and increased incidence of no ossification of hyoid, unilateral/bilateral wavy ribs, and semi-bipartite vertebral centra. At 62.5 mg/kg, there was a statistically significant increase in incomplete ossification of more than one cranial bone. However, in the absence of any other skeletal findings, it was concluded that this effect was due to retarded development, rather than permanent damage, and consequently was not treatment-related. At 12.5 mg/kg, there was a statistically significant increase in the incomplete ossification ..of more than one facial bone, but in the absence of an effect on intermediate dose animals for this finding, and the lack of any other findings, this was not considered to be treatment-related.

Method: OECD 59B (1982)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97.2%
Remarks: No deviations from protocol noted
Reference: Monsanto SP-93-46, SafePharm Laboratories 1994
Reliability: (1) Valid without restriction

Type: Other: Preliminary Developmental Toxicity Screen
Species/strain: Mice, CD-1
Sex: Female
Route of Administration: Oral gavage
Exposure period: Day 6-13 of gestation
Frequency of treatment: Once daily
Post exposure observation period: Until postnatal Day 3
Duration of the test:
Doses: 800 mg/kg bw in corn oil vehicle

Control group: Yes
Concurrent vehicle
Results: Maternal mortality was 48/50 animals. Evaluation and/or classification of the test compound as a potential developmental toxin was impossible due to inadequate maternal survival.
Method: Chernoffavlock Experimental Protocol, 1986
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity 'commercial grade'
Remarks: The Chernoffavlock Experimental Protocol, in which pregnant mice are dosed midterm and then allowed to deliver, was used for this investigation of 60 industrial chemicals as a developmental toxicity screening tool. Testing was performed in NIOSH and contract laboratories. Initially, nonpregnant CD-1 mice were given 10 ml/kg of the test compound orally in corn oil for eight days to determine the 10% lethal dose. Then pregnant mice were orally dosed on gestation days 6-13. The number of liveborn pups, their birth weight, growth and survival to three days of age were used as indices of potential developmental toxicity. The results were tabulated according to maternal and neonatal response variables.
Reference: Hardin, B.D. et al., 1987
Reliability: (4) Unassignable – data from a secondary literature source

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

B. Toxicodynamics, toxicokinetics

Type: Metabolism .
Remarks: The biological fate of the test compound was in rabbits was examined via chromatographic and mass spectromic methods. Doses were 45 mg/kg bw by i.v. or 90 mg/kg/bw intraduodenal. After i.v. injection, the test compound was rapidly eliminated from plasma. Within five hours, 25% of the dose accumulated in the liver, predominately as glucuronide. Low plasma level after intraduodenal application. Within two hours, 22% of the dose accumulated in the liver, 22% of the dose accumulated in the liver, predominately as glucuronide. The test compound and its glucuronide were excreted slowly in urine and bile.
References: Saito, H. et al., 1980

Type: Metabolism .
Remarks: When rat liver microsomes were incubated with the test compound, the content of cytochrome P-450 and the activity of ethoxycoumarin decreased.
References: Zitting, A. 1982

Type: Adsorption .
Remarks: There was no skin penetration after immersing the tails of mice three-quarters into a 50:50 solution of the test compound in oil.
References: Stasenkova, K.P., 1970

Type: Biochemical or Cellular Interactions .
Remarks: Incubation with the test compound caused rapid oxidation of purified human hemoglobin.
References: Williamson, D. et al., 1981

*** 5.11 EXPERIENCE WITH HUMAN EXPOSURE**

Type: Biological Monitoring
Results: Urinary excretion of the test compound (IPPD) was analyzed in sixteen press operators with occupational exposure at a rubber curing worksite. A total of twenty-two urine samples were collected from each worker at the beginning and end of each workday over a 2-week period. Samples were analyzed within 24 hours of collection via HPLC using a method of extraction which resulted in 90% recovery of IPPD. Rapid excretion of IPPD occurred during the working day, with mean levels of IPPD in uring samples collected before and after shifts of 19.55ug/l and 83.57ug/l, respectively. A total of 4.4% of before-work samples and 28.7% of the after-work samples showed no detectable IPPD. A second slow component of excretion was observed during the week, with mean concentrations in before-shift samples rising from 10.8ug/l to 25.8ug/l between the beginning and the end of the work week. In a skin absorption experiment, with one of the authors as subject, one hand was immersed in water containing IPPD for 90 minutes. IPPD levels in the test subject's urine were measured at 0, 3, 5, and 10.5 hours after exposure, and were found to be 0, 100, 350 and 570ug/l, respectively. The excretion rate than dropped with three consecutive slopes and ceased completely seven days after exposure. The authors concluded that the kinetics of excretion of IPPD in workers exposed daily to this compound has two different components, an initially rapid one, followed by a slow one, and that there are three different components of excretion kinetics after skin absorption with half-times of 3, 7 and 24 hours.
Reference: Scansetti, G. et al., 1987

Type: Immunotoxicity
Results: People who had previously demonstrated sensitivity to IPPD by patch testing were evaluated for HLA antigens. There were no differences between IPPD-sensitive individuals and a control population with regard to class 1 HLA antigens, but LLA-Dw antigens were present with a higher frequency in IPPD-sensitive persons. According to the authors, this latter finding indicates that there may be a genetic predisposition for some individuals to develop IPPD sensitivity.
Reference: Hegye, E. et al., 1993

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793-24-8
1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl-

Molecular Weight: **268.4**
Molecular Formula: **C18-H24-N2**

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance: Organic
B. Physical State: Dark purple-brown opaque liquid
C. Purity: 96-99 % Typical for Commercial Products

1.2 SYNONYMS

Santoflex® 6PPD
Santoflex® 13
Vulkanox® 4020
Accinox® ZC
Antozite® 67P
Flexzone® 7P
Wingstay® 300
UOP® 588
Permanax 6PPD
6PPD
p-Phenylenediamine, N-(1,4-dimethylbutyl)-N'-phenyl-

1.3 IMPURITIES

4-Aminodiphenylamine (CAS# 101-54-2) <1.5%

1.4. ADDITIVES

None

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	45°C (initial)
Decomposition:	No
Sublimation:	No
Method:	FF83.9-1 Initial and Final Melting Point of Organic Compounds.1996.
GLP:	Yes
Remarks:	Capillary method
Reference:	ASTM D-1519 / Flexsys Physical Methods of Analysis
Reliability:	(1) Valid without restriction

Value:	46°-50.1°C (initial to final)
Decomposition:	No
Sublimation:	No
Method:	Instrumental – Differential Scanning Calorimeter, 2001
GLP:	Yes
Remarks:	None
Reference:	Flexsys AP# 2001.150, 2001
Reliability:	(1) Valid without restriction

*2.2 BOILING POINT

Value:	>350°C
Pressure:	1013 hPa
Decomposition:	No
Method:	Instrumental – Differential Scanning Calorimeter, 2002
GLP:	Yes
Remarks:	Sample was run from ambient temperature to 350° at 10°/minute Straight baseline with no endotherm after melt, indicating thermal stability.
Reference:	Flexsys AP# 2002.118, 2002
Reliability:	(1) Valid without restriction

Value:	369.67 °C
Pressure:	1013 hPa
Method:	MPBPWIN v1.40 / Adapted Stein & Brown Method
GLP:	No
Remarks:	Estimation method based on molecular structure and measured values for melting point and water solubility.
Reference:	EPIWIN/MPBPWIN v1.40
Reliability:	(2) Valid with restrictions – modelling data

†2.3 DENSITY

Type:	Density
Value:	1.000
Temperature:	15 °C
Method:	FF97.8-1 Flexsys Standard Method 1997
GLP:	Yes
Remarks:	Density of solids by displacement
Reference:	Flexsys Physical Methods of Analysis
Reliability:	(1) Valid without restriction

***2.4 VAPOUR PRESSURE**

Value:	0.08533 hPa @ 162°C 0.33330 hPa @ 180°C 1.33332 hPa @ 200°C 5.33280 hPa @ 227°C
Method:	measured
GLP:	No data
Remarks:	Pressures determined for expected manufacturing process temperatures
Reference:	Monsanto Report # MAK004, January, 1983
Reliability:	(2) Valid with restrictions – lack of method detail

Value:	0.0000352 hPa
Temperature:	25°C
Method:	MPBPWIN v1.40
GLP:	No
Remarks:	Estimation method based on molecular structure and measured values for melting point and water solubility.
Reference:	EPIWIN/MPBPWIN v1.40
Reliability:	(2) Valid with restrictions – modelling data

*2.5 PARTITION COEFFICIENT $log_{10}P_{ow}$

Log Pow:	4.68
Temperature:	Not Applicable
Method:	calculated SRC LogKow (KowWin) Program 1995
GLP:	No
Remarks:	None
Reference:	Meylan, W.M. and. P.H. Howard, 1995 J. Pharm. Sci. 84: 83-92
Reliability:	(2) Valid with restrictions – modelling data

Log Pow:	4.77
Temperature:	25 °C
Method:	Measured GC Method for Pow
GLP:	No
Remarks:	1% and .01% solutions in 100 ml n-Octanol added to500 ml water. Shaken for 48 hours, equilibration for several days. Equilibration performed in the dark to preclude photodegradation. Analysis via gas chromatography to determine Pow; Pow = 59000 +/- 34000. Good agreement with calculation method listed above.
Reference:	Monsanto ES-78-SS-20, 1978
Reliability:	(2) Valid with restrictions – lack of method detail

*2.6 WATER SOLUBILITY

A. Solubility

Value:	1.1 ppm
Temperature:	23 °C
Description:	Of very low solubility
Method:	Saturated Solution / Solvent Extraction / GC Analysis
GLP:	No data
Remarks:	CH2Cl2 solvent, 96% recovery at 1 ppm. Equilibrated w/out light
Reference:	Monsanto ES-78-SS-20 MIC Environmental Science Dec. 1978
Reliability:	(1) Valid without restriction

Value:	1.86 mg/l
Temperature:	25 °C
Description:	Of very low solubility
Method:	Saturated Solution / Solvent Extraction / GC Analysis, 1986
GLP:	Yes
Remarks:	Preliminary solubility study for Phase I Hydrolysis
Reference:	Monsanto ABC 32304, Analytical Bio-Chemistry Labs, 1986
Reliability:	(1) Valid without restriction

B. pH Value, pKa Value

2.7 FLASH POINT

Value:	204 °C
Type:	Cleveland Open Cup

Method: ASTM D 92-96
Reference: Flexsys America Data, Test Method for Flash Points and Fire Points by Cleveland Open-cup Apparatus, ASTM D 92-96
Reliability: (1) Valid without restrictions

2.11 OXIDISING PROPERTIES

†2.12 OXIDATION: REDUCTION POTENTIAL

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

B. Other data – Henry's Law Constant

Results: 3.36E-009 atm-m3/mole
Remarks: Calculated at 25°C using water solubility of 1.86 mg/l and melt point of 50.1°C
Reference: EPIWIN/HENRYWIN v3.10
Reliability: (2) Valid with restrictions – modelling data

3. <u>ENVIRONMENTAL FATE AND PATHWAYS</u>

***3.1.1 PHOTODEGRADATION**

Type: Air
Indirect Photolysis:
Type of sensitizer: OH
Concentration of sensitizer: 156000 molecule/m3
Rate constant (radical): 226.4928E-12cm^3/molecule-sec
Degradation: 50% after 0.567 hours
Method: calculated
AOP Program v1.90, 2001
GLP: No
Test substance: Other (calculated)
Reference: EPIWIN/AopWin v1.90
Reliability: (2) Valid with restrictions – accepted calculation method

***3.1.2 STABILITY IN WATER**

Type: Abiotic (hydrolysis) Phase I Study
Half life: Not determined
Degradation: 93% at pH 7.0 and 25°C after 24 hours exposure time
99% at pH 7.0 and 25°C after 7 days
Method: Extraction, ABC Protocol M-8305 (1985)
GLP: Yes
Test substance : As prescribed by 1.1-1.4, purity: >95%
Remarks: Primary stock solutions of 1.00 mg/l of the test compound were prepared in nanograde acetone. Subsequent dilutions for spiking and gas chromatography standards were also prepared in nanograde acetone. Test samples were extracted with three 75ml portions of methylene chloride. The extracts were dried by passing them through a funnel containing anhydrous sodium sulfate. No test substance detected at seven days. Hydrolysis

products identified by GC analysis and confirmed by GS/Mass Spectrometry as test compound (1%), 4-hydroxydiphenylamine (69%) and Benzoquinoneimine-n-phenyl (29%). The Benzoquinoneimine-n-phenyl is the oxidized form of 4-hydroxydiphenylamine (CAS# 122-37-2, C12-H11-N-O). The amine portion of the test compound molecule was not isolated, nor was it apparent from the GC-MS spectra. It was postulated that the amine portion might be present in the hydrolysis water layer, indicating that the linkage was cleaved at the aromatic carbon-nitrogen bond.

Reference: Monsanto #32304, Analytical BioChemistry Labs, March, 1986
Reliability: (1) Valid without restriction

Type: Abiotic (hydrolysis) Phase II Study
Degradation: 73.3% @ pH 5 (light) after 26.7 hours
51.1% @ pH 5 (dark) after 54.3 hours
66.7% @ pH 7 (light, DI water) after 5.7 hours
64.3% @ pH 7 (dark, DI water) after 6.3 hours
85.6% @ pH 7 (light, well water) after 3.7 hours
69.8% @ pH 7 (dark, well water) after 5.7 hours
90.9% @ pH 9 (light) after 6.7 hours
90.4% @ pH 9 (dark) after 6.7 hours
Method: Extraction, ABC Protocol M-8305 (1985)
GLP: Yes
Test substance : As prescribed by 1.1-1.4, purity: >95%
Remarks: The role of artificial sunlight in the degradation of the test compound in water at pH 5, 7 and 9 was investigated by exposing split samples to either illumination from a mercury vapor lamp or wrapped in aluminium foil as a dark control. The test compound appeared to degrade in a first order rate with respect to time, when correlation coefficients ranging from -0.910 through -0.996 for the hydrolysis curves when the natural log of the amount of test compound recovered was plotted versus time. The test compound hydrolyzed most rapidly at pH 7 in both deionized (DI) water and well water, with the rate for well water slightly faster than for DI water. The slowest hydrolysis rate was for pH 5 (dark) at approximately 75% of the rate for pH 7 well water. The pH 9 reaction rate was similar to that of pH 7 (both DI and well water). The hydrolysis products were identified via a GC/Mass Spectroscopy method as benzoquinoneimine-N-phenyl and 4-hydroxydiphenylamine in all cases.
Reference: Monsanto #32579, Analytical BioChemistry Labs, July, 1986
Reliability: (1) Valid without restriction

Type: Abiotic (hydrolysis) Phase III Study
Half life: 36.9 hours
Degradation: 60% @ pH 9.0 after 48 hours
Method: Extraction, ABC Protocol M-8305 (1985)
GLP: Yes
Test substance : As prescribed by 1.1-1.4, purity: >95%
Remarks: The objective of this study was to determine the hydrolysis rate of the test compound in simulated gastric juice (2.0 g sodium

chloride, 3.2 g pepsin, 7.0 ml concentrated HCl plus deionized water for a final volume of 1 liter and a pH of 9.0). The test compound was introduced into simulated gastric juice and monitored over a 48-hour test period. During the 48-hour period, approximately 60% of the test compound hydrolyzed. From the data gathered, a hydrolysis rate constant of -0.0188 and a half-life of 36.9 hours were observed. The approximate solubility of the test compound in simulated gastric juice was determined to be 173 mg/l. Hydrolysis products were identified by GC/Mass Spectroscopy. The major hydrolysis product observed was aniline. A trace of two intermediate hydrolysis products, Benzoquinoneimine-N-phenyl and N-1,3-dimethylbutylamine-p-phenol were observed in the reaction samples. From these observations, it is believed that quinone, as well as methyl pentane, are also hydrolysis products.

Reference: Monsanto #32581, Analytical BioChemistry Labs, February, 1986

Reliability: (1) Valid without restriction

*3.2 MONITORING DATA (ENVIRONMENTAL)

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION

*3.3.1 TRANSPORT

Type: Volatility
Media: Water
Method: Calculation from EPIWIN VP/WS 2001
Results: Volatilization half-life from model river: 2.855E+005 hours
Volatilization half-life from model lake: 3.114E+006 hours
Volatilization Constant from water: 3.36E-009 atm-m3/mole
Remarks: Model river = 1 m deep flowing at 1 m/sec and wind velocity of 3 m/sec.
Model lake = 1 m deep flowing at 0.05 m/sec and wind velocity of 0.5 m/sec.
Calculation based on molecular structure and melt point of 50.1°C and water solubility of 1.86 mg/l
Reference: EPISUITE/EPIWIN 2001
Reliability: (2) Valid with restrictions – modelling data

*3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota-sediment-soil-water
Method: Fugacity level III
Results:

	Mass Amount (%)	Half-life (hrs)	Emissions (kg/hr)
Air	0.0264	1.13	1000
Water	19.6	900	1000
Soil	68.1	900	1000
Sediment	12.2	3.6E+003	0

Persistence time estimated at 941 Hours

Remarks: Calculation based on molecular structure and melt point of 50.1°C and water solubility of 1.86 mg/l.
Reference: EPISUITE/EPIWIN v3.10
Reliability: (2) Valid with restrictions – modelling data

***3.5 BIODEGRADATION**

Type: aerobic
Inoculum: adapted
Concentration of the chemical: 1.002 mg/L related to test substance
Medium: water
Degradation: 40% after 1 hour
57% after 2 Hours
61 % after 2.5 Hours
67% after 3 Hours
62% after 4 Hours
74 % after 5.0 Hours
97 % after 22 Hours
Results: readily biodegradable
Method: Primary Biodegradation by Natural Water Die-Away Test, Dixon, Hicks and Michael, 1981
GLP: Yes
Test substance As prescribed by 1.1-1.4, purity: >96%
Remarks: Tests run in Mississippi river water collected on 4/27/81 at the St. Louis waterfront and on purified Milli-Q water. A portion of the river water was sterilized by membrane filtration. A second portion was filtered through glass wool to remove particulates without elimination of the active biomass. The short half-lives in both systems suggest that the compound should not persist in natural aquatic environments.

Rate of test substance disappearance in test waters

Time	River water (active)	River water (sterile)	DI water
0 hours	100%	100%	100%
1 hour	60%	85%	100%
2 hours	43%	70%	88%
3 hours	33%	56%	86%
4 hours	38%	49%	80%
5 hours	26%	41%	65%
22 hours	3%	4%	12%

Reference: Monsanto ES-81-SS-52 Environmental Sciences Labs Dec. 1981
Reliability: (1) Valid without restriction

Type: aerobic
Inoculum: adapted
Concentration of the chemical: 30.0 mg/l related to test substance
Medium: water
Degradation: 7.2% of theory CO2 evolution after 32 days
Results: not readily biodegradable
Method: Ultimate Biodegradation by Monsanto Shake Flask Procedure, Gledhill, Appl. Microbiol. 30, 922 (1975)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity:

Remarks:	In the shake flask procedure, 60ml of acclimated bacterial seed is mixed with 440 ml of minimal salts media in a fluted 2-l Erlenmeyer flask. A weighed quantity of the test material is added. The solution is aerated with 70% oxygen in nitrogen. An open reservoir containing 10 ml of 0.2N barium hydroxide is suspended via a glass tube inserted in a rubber stopper. Provisions for removal and addition of the barium hydroxide solution, aeration and sampling are provided. Flasks are agitated on a rotary shaker at 80 rpm, in the dark, and at ambient temperature. Samples are removed at 3, 7, 14, 21, 38 and 35 days for analysis. CO2 values obtained with the control are subtracted from values for the test material. Considering the rapid primary degradation of the test compound in the River Die-Away Test, the failure to obtain significant CO2 evolution suggests formation of more persistent metabolites or degradation products.
Reference:	Monsanto ES-78-SS-28, MIC Environmental Sciences, 1979
Reliability:	(1) Valid without restriction

3.6 BIOACCUMULATION

Species:	Other
BCF:	801.1
Method:	BCFWIN v2.14
GLP:	No
Remarks:	Calculated using measured melt point of 50.1°C and water solubility of 1.86 mg/l
Reference:	EPIWIN/BCFWIN v2.14
Relaibility:	(2) Valid with restrictions – modelling data

Species:	Other
BCF:	490 (+/-170)
Method:	Neely et al., 1974 (Calculation from measured Log Pow)
GLP:	No
Remarks:	Calculation based on measured Log Pow value of 4.77
Reference:	Monsanto ES-78-SS-20, Environmental Sciences, 1978
Reliability:	(2) Valid with restrictions – lack of method detail

4. ECOTOXICITY

*4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type of test:	static Closed -system
Species:	Salmo gairdneri (Rainbow Trout)
Exposure period:	96 Hours
Results:	LC_{50} (24h) = 0.28 mg/l LC_{50} (48h) = 0.18 mg/l LC_{50} (96h) = 0.14 mg/l NOEC = 0.087 mg/l LOEC = 0.10 mg/l
Analytical monitoring:	No
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	No data

Test substance:	As prescribed by 1.1-1.4, purity: >95%.
Remarks:	The test material, in reagent-grade Acetone, was introduced into 15 liters of diluent water in all-glass vessels. Nominal test concentrations (duplicate) were 0, 0.087, 0.10, 0.12, 0.14, 0.16, 0.18, 0.24, 0.42 mg/l, plus a solvent (acetone) control. To each test vessel, 10 rainbow trout, standard length 3.7 cm, were then added. The test fish were not fed 48 hours prior to testing, nor during exposure. No aeration was provided during the test, and temperature was maintained at 12°C. Dissolved oxygen ranged from 9.9 mg/l (93% saturation) to 2.8 mg/l (26% saturation) from beginning to end of exposure, respectively. pH values ranged from 7.0 initially, to 6.8 at the end of the test. Observations and mortality counts were made every 24 hours. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50s and the 95% confidence intervals were calculated from the regression equation.
Reference:	Monsanto BN-76-256 EG&G Bionomics Aquatic Tox Lab 1977
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

Type of test:	static Closed system
Species:	Lepomis machrochirus (Bluegill Sunfish)
Exposure period:	96 Hours
Results:	LC_{50} (24h) = 0.65 mg/l LC_{50} (48h) = 0.45 mg/l LC_{50} (96h) = 0.40 mg/l NOEC = 0.24 mg/l LOEC = 0.32 mg/l
Analytical monitoring:	No
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975)
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: >95%.
Remarks:	The test material, in reagent-grade Acetone, was introduced into 15 liters of diluent water in all-glass vessels. Nominal test concentrations (duplicate) were 0, 0.24, 0.32, 0.42, 0.65 or 1.0 mg/l, plus a solvent (acetone) control. To each test vessel, 10 bluegill, standard length 3.8 cm, were then added. The test fish were not fed 48 hours prior to testing, nor during exposure. No aeration was provided during the test, and temperature was maintained at 22°C. Dissolved oxygen ranged from 8.6 mg/l (98% saturation) to 0.2 mg/l (2% saturation) from beginning to end of exposure, respectively. pH values ranged from 7.2 initially, to 6.7 at the end of the test. Observations and mortality counts were made every 24 hours. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50s and the 95% confidence intervals were calculated from the regression equation.
Reference:	Monsanto BN-76-256 EG&G Bionomics Aquatic Tox Lab 1977
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

Type of test:	flow-through Open system
Species:	Pimephales promelas (Fathead Minnows)
Exposure period:	28 Days
Results:	LC_{50} (2D) = 2.00 mg/l LC_{50} (7D) = 0.35 mg/l LC_{50} (14D) = 0.27 mg/l LC_{50} (28D) = 0.15 mg/l NOEC = 0.066 mg/l LOEC = 0.12 mg/l
Analytical monitoring:	Yes
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, 1975
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >95%.
Remarks:	The fathead minnows were obtained from Pender's Fish Hatchery in Brady, Nebraska. All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. During the holding, acclimation and test periods, fish were fed a standard commercial fish food in the amount equivalent to 3% of body weight. Fathead minnows used had a mean standard weight of 1.3g and a mean standard length of 40.1mm. As a quality check, the fish were challenged with the reference compound Antimycin A prior to testing. The results indicated that the fish were in good condition. A proportional diluter system was used for the intermittent introduction of the test compound and diluent water into the test aquaria. Aerated will water was delivered to the glass aquaria at the rate of 300ml/minute/aquarium, an amount sufficient to replace the 30 liter test volume at least 14 times in each 24-hour period. The test aquaria were maintained at 22°C. Stock solutions were prepared in nanograde acetone. The nominal concentrations of the test compound were 0, 0.066, 0.12, 0.23, 0.45 or 1.0 mg/l plus a solvent (acetone) control. The fish were observed for mortality and abnormal behaviour initially, and then once every 24 hours during the 28 day test period. The actual concentrations of the test substance were analyzed by gas chromatography on days 0, 1, 5, 10, 14, 21 and 28. Thirty (30) fish per concentration level were used. The measured concentrations of the test substance were 0, 0.033, 0.075, 0.16, 0.40 and 1.0 mg/l. The LC50 values and 95% confidence intervals were calculated using the statistical methods of Litchfield and Wilcoxon (1949). Water quality parameters of temperature, dissolved oxygen, pH and ammonia wer measured in the control, low concentration and high concentration throughout the test. All remained within acceptable limits. The dissolved oxygen concentration stayed between 60-100% saturation. The ammonia concentrations remained below toxic levels. The experiment was originally designed to run for 14 days, but was extended to 28. Test results indicated that a lethal threshold concentration was not reached at 28 days. The test compound also appeared to have cumulative toxicity.
Reference:	MonsantoAB-78-121-B Analytical BioChemistry Labs July 1979
Reliability:	(1) Valid without restriction

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test:	static Closed system
Species:	Daphnia magna
Exposure period:	48 Hours
Results:	EC_{50} (24h) = 1.00 mg/l EC_{50} (48h) = 0.82 mg/l NOEC = 0.56 mg/l
Analytical monitoring:	No
Method:	EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, 1975
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >95%
Remarks:	The Daphnia magna used in the test were cultured at the ABC facilities. Adult Daphnia were fed an algae and trout chow mixture daily until 24 hours prior to testing. The bioassay was conducted in 500 ml glass beakers containing 250 ml of ABC well water. During the test, dissolved oxygen concentration ranged from 8.7-7.4 mg/l, pH range was 7.7-8.4, hardness (CaCO3) was <250 mg/l, and alkalinity was <250 mg/l. Vessels were kept in a water bath at 20°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range-finding experiment was carried out to determine the exposure concentrations for the definitive test. Acetone was used as the solvent for the test solutions, and the experiment included both a control and a solvent control (0.01ml). Concentrations (in duplicate) of the test substance were 0, 0.56, 0.75, 1.0, 3.2, 5.6, 7.5 or 10 mg/l. Ten daphnia, first instar less than 24 hours old, were placed in each test chamber. Daphnia in all concentrations were observed once every 24 hours for mortality and abnormal effects. Water quality measurements were monitored throughout the testing and were considered adequate and equivalent to those measurements in the control chamber. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized program developed by Stephan et al. This program calculated the LC50 statistic and its 95% confidence limits using the binomial, the moving average, and the probit tests.
Reference:	Monsanto AB-78-121 Analytical BioChemistry Labs, June 1978
Reliability:	(1) Valid without restriction

Type of test:	static Closed system
Species:	Daphnia magna
Exposure period:	48 Hours
Results:	EC_{50} (48h) = 0.51 mg/l (undegraded test compound) >1.00 mg/l (degraded test compound) NOEC = 0.25 mg/l (undegraded test compound) >1.0 mg/l (degraded test compound)

Analytical monitoring: No
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, 1975, and MIC Environmental Assessment Method for Conducting Acute Toxicity Tests with Daphnia magna, 1980
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: >95%
Remarks: The test compound is known to undergo rapid chemical transformation in water. The purpose of this study was to determine if the toxicity of the test compound to Daphnia magna decreases concurrently with this chemical degradation. The acute toxicity tests were conducted immediately after spiking the test compound into well water, and then again after the test compound was aged for 24 hours in well water. The Daphnia magna used were cultured at the MIC aquatic laboratory. Adult Daphnia were fed a mixture of Purina Trout Chow and alfalfa daily. Daphnids known to be less than 24 hours old were separated from the adults and used for this study. Static toxicity tests were conducted in 250ml beakers containing 200 ml of the test solution. The well water used was from St. Peters, MO. All test vessels were maintained at room temperature. Test solutions were not aerated during the study. Water quality parameters of dissolved oxygen content, pH, temperature, hardness and alkalinity were monitored at initiation (control only) and at termination in the high, middle and low concentrations. The experiment was run in triplicate. Ten daphnids per test vessel were added within 30 minutes after the addition of the test compound. A 1.0 mg/l test solution was made up by pipetting an appropriate amount of test compound dissolved in acetone into 1 liter of water. The maximum acetone concentration was 1 ml/l. The nominal test concentrations were 0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/l for the undegraded test compound and 0, 0.25, 0.5 and 1.0 mg/l for the degraded compound. The LC50 values and 95% confidence intervals were calculated using the statistical method of Litchfield and Wilcoxon (1949). During the two static tests, DO concentrations ranged from 6.4-8.5 mg/l, pH from 7.6-8.3, the average temperature was 22°C, alkalinity was 210-290 mg/l and hardness was 218-274 mg/l. The results indicated that the non-degraded test compound is highly toxic to Daphnia magna, but that degraded material has significantly reduced toxicity.
Reference: Monsanto ES-80-SS-11, MIC Laboratories, 1980
Reliability: (1) Valid without restriction

***4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae**

Species: Selenastrum capricornutum
Endpoint: Biomass and Growth rate
Exposure period: 96 Hours
EC_{50} (24.h) = 2.0 mg/l
EC_{50} (48.h) = 0.5 mg/l
EC_{50} (72.h) = 0.5 mg/l
(Endpoint) EC50 (96.h) = 0.6 mg/l
NOEC = Not determined
LOEC = 0.1 mg/l

Analytical monitoring:	No
Method:	EPA Selenastrum capricornutum Algal Assay Test 1971 Closed -system
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: >95%
Remarks:	The test algae were obtained from the US EPA Environmental Research Laboratory in Corvallis, Oregon. Beginning cell numbers in the test flasks were 1.0 x 10(4) cells/ml. Cultures were incubated at 24°C under approximately 4,300 lux illumination. Triplicate cultures were employed for each of the test concentrations and the control. Test containers were 125ml flasks containing 50ml of test medium. Concentrations for the definitive test were based on the results of a 72-hr range-finding study. These concentrations were 0, 0.1, 0.3, 0.6, 1 or 3 mg/l. Reagent-grade acetone was used to prepare the stock solutions and as the solvent control, maximum volume 0.05 ml acetone. The pH values ranged from 7.5 at the beginning of the study, to 7.3 at the 96-hour mark. There were no other water quality measurements reported in this study. Statistical analysis involved converting each test concentration to a logarithm, and the corresponding percentage decrease of in vivo chlorophyll a or cell numbers was converted to a probit (Finny, 1971). The EC50s and 95% confidence limits were then calculated by linear regression.
Reference:	Monsanto BN-78-362 EG&G Bionomics Sept. 1978
Reliability:	(2) Valid with restrictions – lack of water quality and GLP data

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Type:	LD_{50}
Species/strain:	Rats, Sprague-Dawley Albino
Value:	>5000 mg/kg bw
Sex:	Male and female
# of Animals:	10
Vehicle:	None - undiluted
Doses:	5000 mg/kg bw
Method:	EPA/TSCA Acute Oral Toxicity and the EEC Methods for Determining Toxicity, Part B.1, No. L 251/96 (Limit Test) Sept. 1984
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 97.6%
Remarks:	Following a range-finding study using doses of 500-5000 mg/kg, the test compound was fed to a group of five male and five female rats in a single oral dose of 5000 mg/kg body weight. Males used in this study weighed between 226-267 grams, and females between 220-251 grams. Animal housing and care conformed to AAALAC standards and to those published in the Guide for the Care and Use of Laboratory Animals, NIH Publication No. 86-23. Rats were observed twice daily and weighed weekly. Two males and one female died prior to sacrifice. A gross necropsy

examination was performed on all surviving animals at sacrifice on Day 15. Clinical findings included decreased fecal output, fecal/urine stains, rough coat, piloerection and soft stools. One male and three females showed weight loss; all other animals gained weight. Most notable internal necropsy finding was black, hard material in the stomach contents. Findings in animals that died included discolored mucoid contents throughout the digestive system with reddened mucosa/dark red foci of the stomach.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
5000	2/5	1/5	3/10

Reference: Monsanto PK-91-108 Springborn Laboratories Nov. 13, 1991
Reliability: (1) Valid without restriction

Type: LD_{50}
Species/strain: Rats, Sprague-Dawley Albino
Value: 3580 mg/kg bw
Sex: Male and female
# of Animals: 25
Vehicle: None - undiluted
Doses: 2510, 3160, 3980, 5010 or 6310 mg/kg bw
Method: Single Oral Dose, Younger Laboratories Protocol, 1973
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >96%
Remarks: Five groups of male and female rats (5 animals/dose level) were fed a single oral dose of the undiluted test article warmed to 115°F to liquefy via oral gavage. Male rats had initial average body weights of 210-250 grams: females had initial average body weights of 215-255 grams. Dosages were 2510, 3160, 3980, 5010 or 6310 mg/kg. Clinical signs of toxicity included reduced activity and appetite for two to five days for survivors, and increasing weakness, diarrhea, ocular discharge, collapse and death for decedents in two to eleven days, with most deaths occurring within seven days. Gross autopsy findings on decedents were hemorrhagic areas in the lungs, discolored livers (jaundiced) and acute gastrointestinal inflammation. Survivors were sacrificed after twelve days. All viscera of survivors appeared normal except for a slight discoloration of the liver in a few animals. 95% confidence limits were 3400-3760 mg/kg.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
2510	0/2	1/3	1/5
3160	1/3	1/2	2/5
3980	0/2	3/3	3/5
5010	2/3	1/2	3/5
6310	2/2	2/3	4/5

Reference: Monsanto Y-73-172 Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type:	LD_{50}
Species/strain:	Rabbits, New Zealand Albino
Sex:	Male and female
# of Animals:	4
Vehicle:	None-undiluted
Doses:	3160, 5010, 7940 mg/kg bw
Value:	>7940 mg/kg bw
Method:	Single Dermal Dose, Younger Laboratories Protocol, 1973
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: >96%.
Remarks:	The undiluted test substance was applied to the shaved skin of male and female rabbits for a period of 24 hours, followed by a 14 day recovery period. Males in this study weighed 2.0-2.6 kg, and females weighed 2.1-2.2 kg. Dosages were 3160, 5010 or 7940 mg/kg. The test material was held in place by means of an occlusive wrap of latex rubber and secured by bandaging and elastic tape. The occlusive wrap was removed after 24 hours and the excess material was wiped from the test animal. Clinical signs of toxicity were reduced appetite and activity for three to seven days. All animals survived until sacrifice on Day 14. All viscera appeared normal in all animals.

Dose mg/kg	Mortalities-Male	Mortalities-Female	Combined
3160	---	0/1	0/1
5010	0/1	---	0/1
7940	0/1	0/1	0/2

Reference:	Monsanto Y-73-172, Younger Laboratories, Oct. 10, 1973
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

5.2.1 SKIN IRRITATION/CORROSION

Species/Strain:	Rabbits, New Zealand Albino
Sex:	Male and female
# of Animals:	6
Vehicle:	None - undiluted
Value:	0.0/8.0
Results:	Not Irritating
Classification:	Non-Irritating
Exposure Time:	24 Hours
Method:	Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP:	No data
Test substance:	As prescribed by 1.1-1.4, purity: >96%
Remarks:	0.5 ml of the undiluted test substance was applied to the shaved dorsal areas of six albino rabbits. The test material was applied to the skin under 1" square gauze patches and held in contact with the skin by means of an occlusive wrap of latex rubber secured by bandaging and elastic tape. The occlusive wrap and gauze patches were removed after 24 hours. Dermal irritation was scored by the Draize Method, and results were recorded 24, 48, 72 and 168 hours after topical application.

	The Primary Irritation Index was calculated by averaging the mean scores at 24 and 72 hours. The Primary Irritation Index was found to be 0.0 on a scale of 0.0-8.0.
Reference:	Monsanto Y-73-172, Younger Laboratories, 1973
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

5.2.2 EYE IRRITATION/CORROSION

Species/strain:	Rabbits, New Zealand Albino
Sex:	Male and female
# of Animals:	6
Vehicle:	None - undiluted
Value:	1.2/110.0
Results:	Slightly irritating
Classification:	Non-irritating
Exposure Time:	24 Hours
Method:	Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP:	No data
Test substance:	As prescribed in 1.1-1.4, purity: >96%
Remarks:	0.1 ml of the undiluted test substance was applied to one eye of six albino rabbits. The other eye was not treated and served as a control. The cornea, iris and conjuntivea were examined immediately after treatment, and then at intervals of 1 hour, and at 24, 48, 72 and 168 hours. The Draize Method was used for scoring eye irritation. Immediate findings: slight discomfort. Immediate: slight discomfort At 1 hour: slight erythema, copious discharge At 24 hours: slight erythema in 5 animals, moderate discharge At 48 hours: slight erythema in 5 animals At 72 hours: all animals scored “0” At 168 hours: all animals scored “0” The average Draize score for 24, 48 and 72 hours was calculated for each animal and then averaged over the six animals. The average Draize score was 1.2 on a scale from 0-110.
Reference:	Monsanto Y-73-172, Younger Laboratories, 1973
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

5.3 SKIN SENSITISATION

Type:	Maximization Test
Species/strain:	Guinea Pigs
# of animals:	No data
Vehicle:	Olive oil or vaseline
Dose:	No data
Result:	Sensitizing
Method:	No data
Test substance:	As prescribed by 1.1-1.4, purity: Commercial grade 6PPD
GLP:	No data
Remarks:	50% sensitization (challenge with 0.05% test compound) 90% sensitization (challenge with 0.5% test compound)
Reference:	Herve-Barzin, B. et al, Contact Dermatitis, 1977
Reliability:	(4) Unassignable – data from a secondary literature source

Type:	Skin Patch Test
Species/strain:	Human
# of subjects:	94
Vehicle:	Petrolatum
Dose:	1%
Result:	Not sensitizing
Method:	Modified Draize, 1976
Results:	No skin reactions were noted in a 6-week study on 94 human volunteers. The induction phase consisted of the application of 1% 6PPD in petrolatum to the same site, three times per week for three straight weeks. In the challenge phase, the test article was applied at a previously unpatched site.
Remarks:	None
Reference:	Monsanto MA-78-91, September 19, 1978
Reliability:	(2) Valid with restrictions – age of study, lack of method detail

Type:	Skin Patch Test
Species/strain:	Humans
# of subjects:	50
Vehicle:	Dimethylphthalate
Dose:	50%
Result:	Sensitizing
Method:	Modified Draize, 1976
Results:	6PPD was patch tested on 50 human volunteers at a concentration of 50% w/v in dimethylphthalate. 5 of the 50 subjects showed skin reactions during the 3-week induction phase of the study. 5 of 50 subjects showed skin reactions in the challenge phase
Remarks:	None
Reference:	Monsanto SH-76-8, Industrial Biology Laboratories, 1976
Reliability:	(2) valid with restrictions – age of study, lack of method detail

*5.4 REPEATED DOSE TOXICITY

Species/strain:	Rats, Sprague-Dawley
Sex:	Male/Female
# of animals:	200 (100 male, 100 female)
Route of Administration:	Dietary
Exposure period:	3 Months (90 day)
Frequency of treatment:	Daily
Post exposure observation period:	
Dose:	0, 250, 1000 or 2500 ppm
Control group:	Yes Concurrent no treatment
NOEL:	250 ppm
LOEL:	Not determined
Results:	The test compound was administered in feed to four groups (25/sex/dose) of 6 week old male and female rats at the above levels. Weight range for males at the start of the study was 196.5-229.1 grams. Females weighed 160.8-203.8 grams. Checks for mortality and moribundity were performed twice daily. Detailed observations for signs of toxicity were performed once weekly, as

were body weight and food consumption measurements. Ophthalmic examinations were done twice – at pretest and then just prior to sacrifice. Clinical pathology analyses were performed twice (weeks 6/7 and 13/14) on ten animals/sex/dose. Analyzed were hematology, leukocyte differential, and reticulocyte counts, along with complete blood chemistry. Fifteen animals/sex/dose were sacrificed and subjected to a complete gross pathologic examination at week 6/7. Analysis of the test material stability on rat feed, homogeneity of diet mixtures and dietary level verification were done via gas chromatography (GC). Analyses via GC verified feeding levels of 0, 230, 950 and 2300 ppm. Statistical procedures used to detect statistically significant differences between treated animals and respective controls included Dunnett's Multiple Comparison Test (two-tailed) for body weights, food consumption, non-categorical clinical pathology data and absolute organ weights, Mann-Whitney Test with Bonferroni Inequality Procedure for organ weight/body weight ratios, and Fisher's Exact Test with Bonferroni Inequality Procedure for the incidence of microscopic lesions. All animals survived the length of the study. Signs of toxicity during the study were limited to reduced feed consumption/body weight gain in the high-dose males and females and mid-level males. Anemia, lymphocytopenia and thrombocytosis were present in males and females, primarily at the two highest dose levels. Increases in total bilirubin in males, and total protein, albumin, globulin, calcium and/or cholesterol in both sexes were noted in high and some mid-dose level animals. Increased liver weights were observed at the two highest dose levels. There were no gross or microscopic lesions attributed to consumption of the test material. Females at low dose levels exhibited mild anemia at the interim sampling period, but all recovered by the end of the study. Therefore, the NOEL was considered to be 250 ppm

Method: OECD Guidelines for Testing of Chemicals, Section 412, 1981
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97.1%
Reference: Monsanto ML-85-223 Monsanto Environmental Health Lab May 21, 1987
Reliability: (1) Valid without restriction

Species/strain: Rats, Charles River Albino (COBS)
Sex: Male/Female
# of animals: 40
Route of Administration: Inhalation
Exposure period: 4 Weeks
Frequency of treatment: 6 hr/day, 5 days/week for 4 weeks. (Total = 20 exposures)
Post exposure observation period:
Dose: 0, 50, 250 or 500 mg/m3
Control group: Yes
Concurrent no treatment
NOEL: Not determined
LOEL: 50 mg/m3
Results: Four groups of 5 male and 5 female young adult albino rats were exposed to either zero, low, intermediate or high dust

concentrations of the test article. Test dusts were suspended in streams of clean, dry air, and introduced through the top center of exposure chambers and exhausted out the bottom. GC analytical testing confirmed concentrations and total weight of test dusts. Observations were made with respect to incidence of mortality, reactions displayed and body weight effects. Hematologic and clinical chemistry studies and urinalyses were conducted on all test and control animals on Day 23. All but one animal survived until sacrifice on Day 28. A complete set of organs and tissues was removed from each animal and preserved in formalin. Histopathologic studies were conducted on selected tissues and organs from the control and high concentration groups. Weights of selected organs were recorded and subjected to statistical analyses. A sample of the airborne dust was collected weekly from the test atmosphere for particle size determination. Statistical calculations were performed via computerized programs that utilized Scheffe's Multiple Comparison Test, Tukey's Multiple Comparison Test and analysis of variance. Findings: Hypoactivity was noted in all test groups. Mid and high-dose animals exhibited swollen snouts and scratching. Mean body weights of treated animals compared favorably with those of controls. Results of gross necropsy indicated increased liver and kidney weights of treated animals over those of controls. Lung weights were reduced in high-dose males and mid-dose females. Mid-dose treated males exhibited increased spleen weights. No significant differences were noted in the weights of the brains, gonads and hearts of treated animals when compared to controls. No gross or histopathologic alterations attributed to the test article were observed in any of the treated animals.

Method: Subacute Dust Inhalation Protocol IBT #8562-09721 (Audited)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 97.1%
Reference: Monsanto BTL-76-142 Industrial Bio-Test Labs June 11, 1979
Reliability: (1) Valid without restriction

Species/strain: Rats, Charles River Albino
Sex: Male/Female
# of animals: 400
Route of Administration: Dietary
Exposure period: 2 Years
Frequency of treatment: Daily
Post exposure observation period:
Dose: 0, 100, 300 or 1000 ppm (0, 8, 23 or 75 mg/kg bw/day)
Control group: Yes
Concurrent no treatment
NOEL: 300 ppm
LOEL: 1000 ppm
Results: The test compound was fed at the above doses to groups of 200 male and 200 female rats over a two-year period, beginning when the males were 28 days old and the females 29 days old. Dose levels were verified by GC analysis. Body weight, food consumption, behavior, hematology, blood chemistry and urinalysis results were recorded throughout the study. Complete

gross necropsies were conducted on all animals found dead, on all animals sacrificed in extremis, and on all remaining animals at 24 months.
All organs or tissues with grossly visible lesions were submitted for histologic examination. Statistical reductions in body weight were noted in high-dose males during Weeks 1-5. High-dose females exhibited statistically reduced body weights throughout the study. Body weights and weight gain of the mid- to low-dose animals compared favorably to controls. Frequency and distribution of deaths during the study were similar between treated animals and controls. Gross pathological examination of animals that died during the study did not reveal any relation to death and the test article. There were no unusual behaviors noted in test animals during the study. A significant reduction in erythrocyte counts was noted in high-dose males at 3 months and in high-dose females at 3, 6, and 9 months. However, the same animals had erythrocyte counts similar to controls at all subsequent blood collections. Hemoglobin concentration, while still considered to be within normal range, was statistically reduced for high-dose males at 3, 12 and 18 months. High-dose females exhibited similar reductions at 6, 12 and 18 months. Hematocrit values among high-dose animals were significantly lower than controls, and were at the lower limits at 3 and 12 months for males, and 3, 6 and 12 months for females. Hematocrit values in these animals exhibited a slight increase at 18 and 24 months. Urinalysis studies, which included monitoring of glucose, albumin, microscopic elements, pH and specific gravity, were similar for both treated and control groups throughout the study. Gross pathological examination of animals sacrificed at 24 months revealed similar findings for both treated and control groups. Statistical analysis of absolute organ weights, organ to body weight ratios and organ to brain weight ratios compared favorably across the test and control groups, and were within the range of expected values for albino rats of this age and strain. Histopathological examination of organs and tissue taken from high-dose animals and controls at 24 months revealed no treatment-related lesions. Any lesions noted were from those of naturally-occurring diseases, and were noted in both populations. Microscopic examination of suspect lesions from all sacrificed animals and also those that died during the study. No differences were noted between test and control rats as to the organ system involved, type or classification of neoplasms.

Method: 2-Year Chronic Oral Toxicity IBT Protocol # 622-05400A (1974)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96.9%
Reference: Monsanto BTL-74-26 Industrial Bio-Test Labs Nov. 27, 1978
Reliability: (1) Valid without restriction

*5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

Type: Bacterial Reverse Mutation Assay - Ames

System of testing:	Salmonella typhimurium TA-1535, TA-1537, TA-1538, TA-98, TA-100
Concentration:	0.1, 1.0, 10.0, 100.0 and 500.0 ug/plate
Metabolic activation:	With and without
Results:	
Cytotoxicity conc:	With metabolic activation: 500 ug/plate Without metabolic activation: 500 ug/plate
Precipitation conc:	Not determined
Genotoxic effects:	
	With metabolic activation: Negative Without metabolic activation: Negative
Method:	Ames Plate Test (Overlay method) 1975
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >96%
Remarks:	The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were 10 ug/plate Methylnitrosoguanidine (MNNG), 100 ug/plate 2-nitrofluorene (NF) or 10 ug/plate Quinacrine mustard (QM). Positive controls used for the activation assays were 100 ug/plate 2-anthramine (ANTH), 100 ug/plate 2-Acetylaminofluorene (AAF) or 100 ug/plate 8-Aminoquinoline (AMQ). Dimethylsulfoxide (DMSO) at 50 ul/plate was used as the solvent and the solvent control. Statistical analysis was performed on plate incorporation assay results after transforming revertant/plate values as Log10 (revertants/plate). Analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present. Positive control treatments produced the expected large increases in the frequency of histidine revertants. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.
Reference:	Monsanto BIO-76-227, Litton Bionetics, December 1976
Reliability:	(1) Valid without restriction

Type:	Bacterial Reverse Mutation Assay - Ames
System of testing:	Salmonella typhimurium TA-1535, TA-1537, TA-1538, TA-98, TA-100
Concentration:	0.001, 0.01, 0.1, 1.0 and 5.0 microliters/plate
Metabolic activation:	With and without
Results:	
Cytotoxicity conc:	With metabolic activation: 5.0 ul/plate Without metabolic activation: 5.0 ul/plate
Precipitation conc:	Not determined

Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Ames Plate Test (Overlay method) 1975
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: >96%
Remarks: A hexane extract of the test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were 10 ug/plate Methylnitrosoguanidine (MNNG), 100 ug/plate 2-nitrofluorene (NF) or 10 ug/plate Quinacrine mustard (QM). Positive controls used for the activation assays were 100 ug/plate 2-anthramine (ANTH), 100 ug/plate 2-Acetylaminofluorene (AAF) or 100 ug/plate 8-Aminoquinoline (AMQ). Dimethylsulfoxide (DMSO) at 50 ul/plate was used as the solvent and the solvent control. Statistical analysis was performed on plate incorporation assay results after transforming revertant/plate values as Log10 (revertants/plate). Analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present. Positive control treatments produced the expected large increases in the frequency of histidine revertants. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.
Reference: Monsanto BIO-77-94, Litton Bionetics July 1977
Reliability: (1) Valid without restriction

Type: Bacterial Reverse Mutation Assay - Ames
System of testing: Salmonella typhimurium TA-1535, TA-1537, TA-1538, TA-98, TA-100
Concentration: 0.001, 0.01, 0.1, 1.0 and 5.0 microliters/plate
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 5.0 ul/plate
Without metabolic activation: 1.0 ul/plate
Precipitation conc: Not determined
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Ames Plate Test (Overlay method) 1975
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: >96%
Remarks: A methanol extract of the test compound was evaluated for genetic activity in microbial assays with and without the addition

of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were 10 ug/plate Methylnitrosoguanidine (MNNG), 100 ug/plate 2-nitrofluorene (NF) or 10 ug/plate Quinacrine mustard (QM). Positive controls used for the activation assays were 100 ug/plate 2-anthramine (ANTH), 100 ug/plate 2-Acetylaminofluorene (AAF) or 100 ug/plate 8-Aminoquinoline (AMQ). Dimethylsulfoxide (DMSO) at 50 ul/plate was used as the solvent and the solvent control. Statistical analysis was performed on plate incorporation assay results after transforming revertant/plate values as Log10 (revertants/plate). Analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present. Positive control treatments produced the expected large increases in the frequency of histidine revertants. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: Monsanto BIO-77-93, Litton Bionetics July 1977
Reliability: (1) Valid without restriction

Type: Ames Bacterial Reverse Mutation
System of testing: Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100
Concentration: 0.167, 0.500, 1.67, 5.00, 16.7 and 50.0 ug/plate (triplicate)
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation:
Without metabolic activation:
Precipitation conc: 500 ug/plate
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Revised Method for the Salmonella Mutagenicity Test (1983) Maron, D.M. and Ames, B.N.
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: >96%
Remarks: Stock solutions of the test compound were prepared in DMSO. All tester strains contained a uvrB deletion mutation and an rfa mutation. The cytotoxicity of test article was determined in a screening test on duplicate cultures of TA1538 and TA100 in the absence of an exogenous metabolic activation system (S9 mix). Concentrations tested were 50, 167, 500, 1670 and 5000 ug/plate. Results of the pre-screen indicated that the test compound produced inhibited growth (characterized by a reduced background lawn and/or the presence of pindot colonies). The test

compound precipitated from solution at doses equal to or greater than 500 ug/plate.
In the definitive assay, inhibited growth was observed at concentrations >5.00, both with and without S9 activation. The S9 mixture included 6% (v/v) Aroclor 1254-induced male Sprague-Dawley rat liver homogenate with the appropriate buffer and cofactors. Positive controls evaluated in the absence of S9 were sodium azide at 10 ug/plate (TA1535 and TA100), 9-aminoacridine at 150 ug/plate (TA1537), and 2-nitrofluorine at 5 ug/plate (TA1538 and TA98). 2-Anthramine at 2.5 ug/plate was used in all strains in the presence of S9. Statistical analyses were performed using the computer program developed by Snee and Irr (1981), with significance established at the 95% confidence limit. Revertant frequencies for all doses, in all strains, both with and without metabolic activation were equal to or less than those of the concurrent negative control cultures. All positive and negative control values were within acceptable limits.

Reference: Monsanto PK-91-109, Pharmakon Research Intl. July 1991
Reliability: (1) Valid without restriction

B. NON-BACTERIAL IN VITRO TEST

Type: Mammalian Cell Gene Mutation Assay
System of testing: L5178Y mouse lymphoma cells
Concentration: 0.25, 0.5, 1.0, 2.0, 4.0 or 8.0 ug/ml
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 33 ug/ml
Without metabolic activation: > 4 ug/ml
Precipitation conc: Not determined
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: Clive and Spector, Mutation Research 31:17-29 (1975)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: >96%
Remarks: The test article was evaluated for specific locus forward mutation in the L5178Y Thymidine Kinase (TK) mouse lymphoma cell assay. The cells used are heterozygous for a specific autosomal mutation at the TK locus and are BUdR sensitive. Scoring for mutation was based on selecting cells that have undergone forward mutation from a TK+/- to a TK-/- genotype by cloning them in soft agar with BUdR. Stock solutions were prepared in DMSO. DMSO was used as the negative control. The activation system was mouse liver S-9 mix. Ethylmethanesulfonate (EMS) at 0.5 ul/ml was used as the positive control without activation and Dimethylnitrosamine (DMN) at 0.3 ul/ml was used as the positive control with activation. The reference mutagens and induced mutation frequencies within the expected range. The test article did not induce mutagenesis in either assay.

Conc.	Mutant clones	Viable clones	Mutant frequency x10E-4
Non-Activation			
Solvent Control ---	77.0	116.0	0.6638
EMS 0.50	467.0	106.0	4.4057

Test Compound	0.25	148.0	119.0	1.2437
	0.50	64.0	189.0	0.3386
	1.00	139.0	111.0	1.2523
	2.00	62.0	114.0	0.5439
	4.00	97.0	147.0	0.6599
	8.00	Toxic		
Activation				
Solvent Control	---	66.0	106.0	0.6226
DMN	0.30	193.0	80.0	2.4125
Test Compound	1.00	92.0	127.0	0.7244
	2.00	112.0	105.0	1.0667
	4.00	91.0	150.0	0.6067
	8.00	78.0	104.0	0.7500
	16.00	62.0	72.0	0.8611
	32.00	Toxic		

Reference: Monsanto BIO-76-245 Litton Bionetics May 1977
Reliability: (1) Valid without restriction

Type: Cytogenics Assay
System of testing: Chinese hamster ovary (CHO) cells
Concentration: 5, 10 and 12.5 ug/ml without activation
5, 10, 12.5 and 15 ug/ml with activation
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 20 ug/ml
Without metabolic activation: 20 ug/ml
Precipitation conc: Not determined
Genotoxic effects:
With metabolic activation: Marginal
Without metabolic activation: Marginal
Method: EPA Gene-Tox Review Program, Preston et al., 1981
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96%
Remarks: The test compound was evaluated for its potential to induce chromosomal aberrations in cultured Chinese hamster ovary cells. CHO cells were treated with 5, 10 and 12.5 ug/ml of the test compound for 5 hours in the absence of exogenous activation. In the presence of exogenous activation (Aroclor 1254-induced rat liver homogenate), the cells were treated with 5, 10, 12.5 and 15 ug/ml. In the absence of activation, cells were harvested at 6 hours (5 ug/ml), 12 hours (5, 10 and 12.5 ug/ml), 24 hours (5, 10 and 12.5 ug/ml) and 48 hours (10 and 12.5 ug/ml) after initiation of treatment. In the presence of activation, cells were harvested at 6 hours (5 ug/ml), 12 hours (5, 10, 12.5 and 15 ug/ml), 24 hours (5, 10, 12.5 and 15 ug/ml) and 48 hours (10, 12.5 and 15 ug/ml). The different harvest times were chosen based on the average cell generation times calculated for each treatment condition to allow the detection of effects of treatment at different cell cycle stages. Chi-square analysis and Dunnett's t-test were used for statistical analysis. In the nonactivation study, no statistically significant increases in average structural aberrations per cell were observed at any treatment level, regardless of harvest time. At the 24 hour

harvest time, a significant increase of cells with aberrations at 10 and 12.5 ug/ml as well as a significant dose-response relationship was observed. In the activation study, the percentage of cells with structural aberrations was significantly elevated at 10 ug/ml at the 24 hour harvest time. However, the average structural aberrations per cell at this dose/harvest time was not statistically increased above background ($p>0.05$, Dunnett's t-test) The average structural aberration per cell was increased at 5 ug/ml at 6 hours. However, the percentage of cells with structural aberrations was not significantly elevated. As observed for treatment in the absence of activation, the dose response relationship for the induction of aberrant cells was found to be significant at the 24 hour harvest time. The positive controls, MMS and cyclophosphamide, yielded the expected statistically significant positive responses, indicating the adequacy of experimental conditions. The test compound was concluded to have marginal clastogenicity in CHO cells under experimental conditions. However, because of the low magnitude of response, (0-5% aberrant cells, close to the spontaneous rate) the biological significance of the findings is questionable.

Reference: Monsanto ML-86-125 Monsanto Environmental Health 1987
Reliability: (1) Valid without restriction

Type: CHO/HGPRT Forward Gene Mutation Assay .
System of testing: CHO Cells, clone K1-BH4
Concentration: 0-333 ug/ml (range-finding)
3 to 15 ug/ml with activation (confirmatory)
1 to 5 ug/ml without activation (confirmatory)
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 9 ug/ml
Without metabolic activation: 4 ug/ml
Precipitation conc: Solubility limit of test article = 333 ug/ml
Genotoxic effects:
With metabolic activation: Negative
Without metabolic activation: Negative
Method: CHO/HGPRT Mutation Assay (1979) Hsie, et.al.
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96%
Remarks: The mutagenic potential of the test substance was evaluated in CHO cells for ability to induce forward mutation at the HGPRT gene locus. A range-finding cytotoxicity study preceded a dose-response mutagenicity experiment using different levels of Arochlor1254 rat liver homogenate (S9) concentrations, followed by a confirmatory dose-response mutagenicity experiment. The compound was tested at S9 concentrations up to a cytotoxic dose of 30 ug/ml. Solutions of the test compound were prepared using DMSO as the solvent on the day of treatment. Positive controls used were benzo(a)pyrene and ethyl methane sulfonate for the activation and non-activation assays, respectively. The subclone K1BH4 of CHO cells was obtained from Dr. Hsie of Oak Ridge National Laboratories. CHO cells were plated the day before treatment. Statistical analysis was according to the methos of Snee

and Irr (1981) designed specifically for the CHO/GHPRT mutation assay. Student's t-test was used to compare treatment data to control data. The Snee and Irr analysis also allowed the determination of dose-response relationship as linear, quadratic, or higher order. A computer program obtained from Joe Irr was used. No statistically significant mutagenicity was observed in the two separate experiments. The positive controls yielded the expected positive responses in mutagenicity, indicating the adequacy of the experimental conditions. Therefore, the test substance was not considered to be mutagenic in CHO cells under the experimental conditions.

Reference: Monsanto ML-86-147 Environmental Health Laboratory January 1987

Reliability: (1) Valid without restriction

Type: Unscheduled DNA Synthesis (UDS)
System of testing: Primary rat hepatocyte cultures
Concentration: 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000 and 5000 ug/ml
Results:
Cytotoxicity conc: 50 ug/ml
Precipitation conc: Not determined
Genotoxic effects: Negative
Method: Williams, G.M., Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures, 1977
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96%
Remarks: Acetone (1%) was used as both solvent and diluent. Primary rat liver cell cultures derived from the livers of two adult male rats weighing 254 and 309 grams (13 and 18 weeks old) were used for the preliminary and replicate experiments, respectively. Three controls were incorporated into each UDS assay: a positive control, a negative (solvent) control, and an untreated medium control. The positive control was 2-Acetylaminofluorene (2-AAF), the solvent control was acetone in the preliminary assay and in the replicate assay. The percentage of cells in repair was calculated as the percentage of cells with at least 5 net grains/nucleus. 150 cells were scored for each concentration reported for each experiment. Cytoxicity was observed at 50, 100 500, 1000 and 5000 ug/ml in both the preliminary and replicate experiments. UDS was measured at concentrations of the test compound between 0.1 and 10 ug/ml in both experiments. All collection of data and pooling of slides were done via programs in the VAX 11/782 computer. The net grain counts were negative at each concentration of the test compound, in the solvent control and in the medium control, in contrast to the strong positive response produced by the positive control 2-AAF in both experiments (35.7 net grains/nucleus). These results indicate that the test compound is not a genotoxic agent under the conditions of the *in vitro* rat hepatocyte DNA repair assay.

Treatment	Conc.	NG	SE	Median	%IR
Control/medium	---	- 8.9	2.6	- 8.8	0

Control/solvent	1%	- 5.3	0.6	- 4.4	0
2-AAF ug/ml	3	22.6	4.3	17.6	90
Test Cpd. ug/ml	0.1	- 7.3	0.3	- 6.6	0
	0.5	- 6.8	1.4	- 6.6	0
	1.0	- 9.8	1.1	- 9.9	0
	5.0	- 6.8	2.1	- 5.5	1
	10.0	- 5.9	0.6	- 5.5	0
	50.0	Toxic			

Reference: Monsanto SR-86-140, SRI International, September 15, 1986
Reliability: (1) Valid without restriction

* 5.6 GENETIC TOXICITY IN VIVO

Type: Mammalian Bone Marrow Cytogenetics Assay
Species/strain: Rats, Sprague Dawley
Sex: Male/Female
Route of Administration: Oral gavage in corn oil vehicle
Exposure period: 6, 18 and 30 hours
Doses: 1000 mg/kg
Results:
Effect on mitotic index or P/N ratio: No statistically significant increase in the incidence or number of aberrations
Genotoxic effects: Negative
Method: EPA Health Effects Test Guidelines EPA 560/6-82-09 (1984).
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 96%
Remarks: The test compound was evaluated in a preliminary study at doses of 900, 1300 and 1790 mg/kg bw. Due to the pharmacotoxic signs observed at 900 mg/kg, and the mortalities occurring at the two higher doses, 1000 mg/kg was selected as the maximum tolerated dose. In the definitive test, 65 adult male and 65 adult female rats (5 male and 5 female rats/group) were dosed with the test article in a controlled study. No pharmacotoxic signs were observed immediately after dosing. Prior to colchicine, however, all animals exhibited decreased body tone, diarrhea, abnormal gait, piloerection and brown discoloration around the oral-nasal region and forepaws. The pharmacotoxic signs indicated that the test article was at or near the maximum tolerated dose. Animals from each group and dose level were sacrificed at 6, 18 and 30 hours after dosing. Control groups received either 10 ml/kg bw of vehicle control (corn oil), or 20 mg/kg bw of the positive control cyclophosphamide (CP). Two to three hours prior to sacrifice, each animal was given a single intraperitoneal dose of colchicines at 4 mg/kg bw to arrest dividing cells in metaphase. Bone marrow was sampled at 6, 24 and 48 hours after dosing with the vehicle or the test substance. A single sampling time of +24 hours was used for the positive control group. A total of 500 (if possible) well spread, intact metaphase cells were scored for the presence of chromosome aberration per experimental treatment point (50/animal) by two investigators (25 each/animal). Slides were scored for increases in the proportion of aberrant metaphases by Chi-square analysis and in the frequency of aberrations/cell by a

one-way analysis of variance (ANOVA). No statistically significant increases in the proportion of aberrant cells or aberrations/cell were observed at the 6, 24 and 48 hour time points. No statistically significant differences from the vehicle controls were detected by this analysis in animals treated with the test compound. The positive control group (CP) yielded the expected positive responses, indicating the adequacy of the experimental test conditions for the detection of clastogens. The test compound at 1000 mg/kg was judged negative in its ability to induce structural chromosomal aberrations to the hemopoietic cells of the rat bone marrow under the experimental conditions of this assay.

Reference: Monsanto PK-87-316, Pharmakon Research International 1987
Reliability: (1) Valid without restriction

*5.8 TOXICITY TO REPRODUCTION

Type: Fertility
Species/strain: Rats, Jcl Sprague Dawley Albino
Sex: Male/Female
Route of Administration: Oral gavage in corn oil vehicle
Exposure period: Males: 42 or 49 days
Females: 14 days prior to mating through Day 7 of gestation
Frequency of treatment: Once a day
Post exposure observation period:
Premating exposure period: males: 28 days
females: 14 days
Duration of the test: No data
Doses: 0, 40, 200 or 1000 ppm
Control group: Yes
Concurrent vehicle
NOEL Parental: >1000 ppm
NOEL F1 Offspring: >1000 ppm
Results: Groups of male and female rats were dosed with the test article at the above levels prior to mating. Males and females from the same dose levels were paired. Animals were observed for body weight, weight gain, food consumption, appearance, behavior, copulation index and fertility index during the life phase of the study. Mated females were sacrificed on Day 14 of gestation and the fetuses removed via Cesarean Section. Fetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies as well as developmental variation

General parental toxicity: All animals survived until planned sacrifice. There were no effects of treatment observed on mean body weight, weight gain, appearance, behavior, physical viability, copulation index or fertility index. There were no remarkable findings in gross necropsy or organ weights.

Toxicity to offspring: The number of corpora lutea and implantations, implantation rate, fetal mortality, and number of live fetuses were not affected by the test article.

Method: Fertility Study and Early Embrionic Development to Implantation in Rats, DRL, 1998
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity >98%

Remarks: The test article is being evaluated as a new diagnostic drug of Helicobacter pylori. To this end, several reproductive and developmental toxicity studies have been conducted recently by this laboratory. All reports published to date have indicated that there are no reproductive, developmental or fetotoxic effects of this chemical under the test conditions.

Reference: Developmental Research Laboratories, Dainippon Pharmaceutical Company, Japan, 1998

Reliability: (4) Not assignable – data from a secondary literature source

Type: Fertility
Other: Three Generation Study

Species/strain: Rats, Charles River Albino

Sex: Male/Female

Route of Administration: Oral/Dietary

Exposure period: Premating, throughout mating, gestation and lactation

Frequency of treatment: Daily

Post exposure observation period: Not Determined

Premating exposure period:
F0 – 14 wks (males)
F1-- 14 wks (males)
F2 – 18 wks (males)
F0 – 14 wks (females)
F1 – 14 wks (females)
F2 – 18 wks (females)

Duration of the test:
F0 – 23 wks
F1 – 23 wks
F2 – 26 wks

Doses: 0, 100, 300 or 1000 ppm (8, 23 or 75 mg/kg bw/day)

Control group: Yes
Concurrent no treatment

NOEL Parental: 100 ppm (based on reduced body weight gain)

NOEL F1 Offspring: 1000 ppm

NOEL F2 Offspring: 1000 ppm

Results: The test compound was administered to three successive generations of rats at dose levels of 0, 100, 300 or 1000 ppm. Dose levels were selected on the basis of results from a previous 2-year chronic oral feeding study. The calculation of the dose levels was based on 1 ppm = 0.075 mg/kg/bw. No adverse effects on mating or fertility indices were noted in any of the treated animals. No substance-related histopathological effects were noted at any dose level. Evidence of parental toxicity was present as indicated by reduced body weights of the mid-to high-dose animals only.

General parental toxicity: Reduced body weights and mean body weight gains were noted for the 300 and 1000 ppm males and females. No other treatment-related effects were evident in results of clinical blood chemistry studies and urinalyses results between the control groups and the treated animals.

Toxicity to offspring: No effect on mating and fertility indices, no effect on fetal, pup or adult survival, no effect on behaviour, no substance-related histopathological effects in the F1 and F2 generations.

Method: 3-Generation Reproductive Toxicity IBT Protocol # 622-05400C (1974)
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 99+% active
Remarks: Protocol similar to Monsanto BTL-74-26, Industrial Bio-Test Labs, 1978 (2 Year Feeding Study)
Reference: Monsanto BTL-76-144, Industrial Bio-Test Labs, 1976
Reliability: (1) Valid without restriction

*5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY

Species/strain: Rats, Sprague Dawley
Sex: Female
Route of Administration: Oral gavage in corn oil vehicle.
Duration of the test: 20 days
Exposure period: Days 6-15 of gestation
Frequency of treatment: 1x/day
Doses: 0, 50, 100 or 250 mg/kg/day
Control group: Yes
Concurrent vehicle
NOEL Maternal Toxicity: 50 mg/kg.
NOEL teratogenicity: >250 mg/kg
Results: Four groups of 25 bred female rats were dosed with the test article at 0, 50, 100 and 250 mg/kg/body weight. Dosages were determined in a preceding range-finding study. Survival was 100% in all groups. Throughout gestation, all animals were observed 2x/day for appearance, behavior, body weight and food consumption. On Day 20, all test animals were sacrificed and the fetuses removed via Cesarian Section. Fetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies as well as developmental variation

Maternal general toxicity: Clinical signs noted in the mid- to High-dose groups included salivation prior to dosing, soft stool, diarrhea and green fecal discoloration. Maternal body weights and weight gain were comparable in all groups. No morphopathological changes which could be attributed to the test article were observed in any of the treated animals

Pregnancy/litter data: No abortions or premature deliveries occurred in any test group.

Foetal data: No differences that could be associated with the test article were observed between the control group and the treated groups with respect to number of viable fetuses, early and late resorptions, fetal sex ratios or fetal weights. The types of malformations and the frequency of such mutations occurring during this study were not those indicative of a teratogenic response.

There was a small, non-statistically significant increase in the incidence and number of skeletal variations in the treated groups. However, these were judged to be common developmental variations of this species and have been observed to occur with similar incidence in the historical data.

Method: Teratology – Principles and Techniques, J.G. Wilson, 1965
GLP: Yes

Test substance:	As prescribed by 1.1-1.4, purity: >97%
Remarks:	Not teratogenic or embryo/fetotoxic under test conditions. This was a follow-up study to a range-finding study (Monsanto WI-85-304) that noted excessive maternal toxicity at dose levels of 2000, 1000 and 600 mg/kg/day, with clinical signs of toxicity in the 300 mg/kg/day group. Intrauterine survival was not affected at the 100 and 300 mg/kg/day dose levels.
Reference:	MonsantoWI-86-363 WIL Research Laboratories October 1987
Reliability:	(1) Valid without restriction

Species/strain:	Rabbits, New Zealand Albino
Sex:	Female
Route of Administration:	Oral in gelatin capsules
Duration of the test:	Post observation – sacrifice on gestation day 29
Exposure period:	Days 6-18 of gestation
Frequency of treatment:	once a day
Doses:	0, 10 or 30 mg/kg bw/day
Control group:	Yes Concurrent vehicle - empty gelatin capsule
NOEL Maternal Toxicity:	30 mg/kg bw
NOEL teratogenicity:	30 mg/kg bw
Results:	Maternal body weight loss and mortality were comparable to that of the controls. There were no treatment-related gross lesions noted at necropsy. There was a slight increase in the number of resorption sites per 100 implantation sites in the high-dose group (38.6%) when compared to controls (31.4%). The resorption sites per 100 implantation sites were at the high end of the range for control New Zealand Albino rabbits used in similar teratogenic studies conducted at this test laboratory. The number of live young per 100 implantation sites for the low-dose group (48.3%) and for the high-dose group (38.6%) were moderately decreased when compared to the controls (68.6%). There was no increase in the incidence of external, visceral or skeletal abnormalities. Treatment of pregnant albino rabbits during the period of organogenesis with either 10 or 30 mg/kg of the test compound did not produce any abnormal fetal development that could be attributed to exposure to the test material.
Method:	Teratology – Principles and Techniques, J.G. Wilson, 1965
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: >96%
Remarks:	All young were examined by careful dissection. Particular attention was paid to any differences in size, shape and orientation of the major organs and blood vessels. An examination of skeletal tissue was then performed employing a modified method for the demonstration of skeletal tissues in embryos as described by Hurley (1965).
Reference:	Monsanto BT-76-146, Industrial Bio-Test Laboratories, 1978
Reliability:	(1) Valid without restriction

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

B. Toxicodynamics, toxicokinetics

* 5.11 EXPERIENCE WITH HUMAN EXPOSURE

Results:	Cross sensitization in rubber workers exposed to various members of the PPD family have been reported. Anecdotal evidence suggests that this class of compounds has a high potential for skin sensitization with prolonged and repeated exposures of sensitive individuals
Remarks:	Occupational eczema study – 6PPD and IPPD exposures
Reference:	B. Herve-Bazin, H, et al. Contact Dermatitis 3, 1-15 (1977)

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COURTNEY M. PRICE
VICE PRESIDENT
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September 8, 2003

Via U.S. Mail and e-mail

Marianne Lamont Horinko
Acting Administrator
U.S. Environmental Protection Agency (EPA)
P.O. Box 1473
Merrifield, VA 22116

Re: Rubber and Plastic Additives (RAPA) Panel
HPV Chemical Challenge Program Submission
Substituted p-Phenylenediamines Category
Revised Robust Summary for CAS No. 68953-84-4

Dear Ms. Horinko:

On July 17, 2003 the RAPA Panel[1] of the American Chemistry Council submitted a revised test plan and revised robust summaries for the Substituted p-Phenylenediamines category. The category includes five of the 37 chemicals RAPA is voluntarily sponsoring in the Program. These revised documents have been posted on EPA's HPV Challenge Program web site (http://www.epa.gov/chemrtk/sbphnyld/c13383tc.htm).

It has recently come to our attention that one of the posted revised robust summary files (for 1,4-Benzenediamine, N,N'-mixed Ph and toyl derivative; CAS number 68953-84-4) incorrectly indicates that the biodegradation study for the chemical was conducted under anaerobic conditions. In fact, the study was conducted under aerobic conditions. As this mistake affects interpretation of results of the study, we request that the robust summary file for CAS number 68953-84-4 submitted on July 17 be replaced with the attached corrected file. The test plan submitted on July 17 is not affected by this change.

This submission is also being sent electronically to the following e-mail addresses: Oppt.ncic@epa.gov and Chem.rtk@epa.gov. If you require additional information, please contact the RAPA Panel's technical contact, Dr. Anne P. LeHuray at (703) 741-5630 or *anne_lehuray@americanchemistry.com*.

Sincerely yours,

Attachment

[1] The RAPA Panel includes the following member companies: Alco Chemicals; Bayer Polymers LLC.; Ciba Specialty Chemicals Corporation; Crompton Corporation; Eliokem, Inc.; Flexsys America L.P.; The Goodyear Tire & Rubber Company; The Lubrizol Corporation; Noveon, Inc.; and, R.T. Vanderbilt Company, Inc.