Chemical Name: Chloronitrobenzenes Category
Submitter: Solutia

The following chemicals are included in the category:

CHEMICAL NAME	CASRN
Benzene, 1-Chloro-2-Nitro-	88-73-3
Benzene, 1-Chloro-3-Nitro-	121-73-3
Benzene, 1-Chloro-4-Nitro-	100-00-5

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:

201- 14392

NCIC HPV
Sent by: Mary-Beth Weaver

04/09/2003 09:37 AM

To: Matthew Moran/DC/USEPA/US@EPA
cc: NCIC HPV@EPA
cc: NCIC HPV@EPA
Subject: IMPORTANT!! There are several test plans that came to the ChemRTK mail box that were never scanned by the NCIC and sent to us.

"Johannsen, Frederick R" <frjoha@solutia.com> on 12/13/2002 02:11:02 PM

To: Rtk Chem/DC/USEPA/US@EPA
cc: "Downes, James E" <jedown@solutia.com>
Subject: HPV Submission for Category: Chloronitrobenzenes

Attached you will find a cover letter summarizing this submission, including identification of the various attachments to this file. All pertain to the HPV submission we are making for the chemical category Chloronitrobenzenes.

<<HPVChloronitrobenzenes.doc>> <<HPVChloronitrobenzenestrans.doc>> <<mncb.rtf>> <<oncb.rtf>> <<pncb.rtf>>

As always, we would appreciate return confirmation of your receipt of this transmission.

Sincerely,

Frederick R. Johannsen
Solutia Inc.

- HPVChloronitrobenzenes.doc
- HPVChloronitrobenzenestrans.doc
- mncb.rtf
- oncb.rtf
- pncb.rtf

Solutia Inc.
575 Maryville Centre Drive
St. Louis, MO 63141

P.O. Box 66760
St. Louis, MO 63166-6760

December 13, 2002

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

Attn: Chemical Right-to-Know Program
In re: HPV Challenge Program
AR-201

Benzene, 1-Chloro-2-Nitro-
CAS Number 88-73-3

Benzene, 1-Chloro-3-Nitro-
CAS Number 121-73-3

Benzene, 1-Chloro-4-Nitro-
CAS Number 100-00-5

Solutia, Inc., Company Registration Number , is pleased to submit the attached Test Plan and Robust Summaries for the Category Chloronitrobenzenes (consisting of Benzene, 1-Chloro-2-Nitro- with CAS No. 88-73-3, Benzene, 1-Chloro-3-Nitro- with CAS No. 121-73-3 and Benzene, 1-Chloro-4-Nitro- with CAS Number 100-00-5) as a part of our commitment to the EPA High Production Volume Challenge Program (AR-201).

The attached files are:

1. This cover letter in MS Word 2000
2. Category Test Plan in MS Word 2000
3. Robust Summaries (IUCLID format) for all three chemicals in this Category in MS Word 2000

The complete matrix of SIDS data elements, including physical/chemical properties and results of biological and toxicology studies, indicate that no additional testing is required.

Please contact me at 314-674-8815 if there are any questions relating to this submission.

Sincerely,

Frederick R. Johannsen

HIGH PRODUCTION VOLUME (HPV)

CHEMICAL CHALLENGE PROGRAM

TEST PLAN

For the

CHLORONITROBENZENE CATEGORY

CAS Number 88-73-3; Benzene, 1-Chloro-2-Nitro-

CAS Number 121-73-3; Benzene, 1-Chloro-3-Nitro-

CAS Number 100-00-5; Benzene, 1-Chloro-4-Nitro-

Prepared by:

Solutia Inc. Registration No.

575 Maryville Centre Drive,
St. Louis, Missouri 63141

RECEIVED
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EXECUTIVE SUMMARY

Solutia Inc. voluntarily submits the following Category Justification, Screening Information Data (Robust Summaries) and Test Plan for review under the Environmental Protection Agency's High Production Volume (HPV) Chemicals Challenge Program. The category, entitled "Chloronitrobenzenes" consists of three members, Benzene, 1-chloro-2-nitro-, also known as o-Chloronitrobenzene (CAS No. 88-73-3), Benzene, 1-chloro-3-nitro-, also known as m-Chloronitrobenzene (CAS No. 121-73-3), and Benzene, 1-chloro-4-nitro-, also known as p-Chloronitrobenzene (CAS No. 100-00-5). This category is justified on the basis of chemical structure similarity, as well as similarity of basic screening data, as provided in an initial assessment of physico-chemical properties, environmental fate and human and environmental effects.

A substantial amount of data exists to evaluate the potential hazards associated with this Category of chemicals. Use of key studies available from data already developed or derived from recommended estimation models provide adequate support to characterize each Endpoint in the HPV Chemicals Challenge Program without the need for additional testing.

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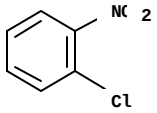
TEST PLAN FOR CHLORONITROBENZENES

I. INTRODUCTION AND IDENTIFICATION OF CATEGORY MEMBERS

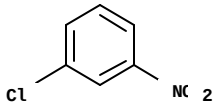
Under EPA's High Production Volume (HPV) Chemicals Challenge Program, Solutia Inc. has committed to voluntarily compile basic screening data on three chemicals of similar structure, namely Benzene, 1-chloro-2-nitro (known as o-chloronitrobenzene or ONCB; CAS no. 88-73-3), Benzene, 1-chloro-3-nitro (known as m-chloronitrobenzene or MNCB; CAS no. 121-73-3) and Benzene, 1-chloro-4-nitro (known as p-chloronitrobenzene or PNCB; CAS no. 100-00-5). Solutia Inc. believes that a category of Chloronitrobenzenes is scientifically justifiable. The data included in this Category involve physicochemical properties, environmental fate, and human and environmental effects of the chemicals in this Category, as defined by the Organization for Economic Cooperation and Development (OECD). Most of the information provided comes from existing data developed on behalf of Solutia Inc., or its predecessor Monsanto Co., much of which has already been submitted to the US EPA under auspices of sections of the Toxic Substances Control Act and is available through TSCATS; additional information can be found in the published scientific literature or from recommended estimation models. This submission fulfills Solutia's obligation to the HPV Challenge Program for these three chemicals.

A. Structure and Nomenclature

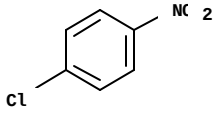
The members of this family of Chloronitrobenzenes, include the following chemicals:



a. Benzene, 1-chloro-2-nitro-
CAS No. 88-73-3
Synonyms: o-Nitrochlorobenzene; o-Chloronitrobenzene; ONCB



b. Benzene, 1-chloro-3-nitro-
CAS No. 121-73-3
Synonyms: m-Nitrochlorobenzene; m-Chloronitrobenzene; MNCB;



c. Benzene, 1-chloro-4-nitro-
CAS No. 100-00-5
Synonyms: p-Nitrochlorobenzene; p-Chloronitrobenzene; PNCB;

B. Manufacturing & Use

Members of the Chloronitrobenzenes Category, p-nitrochlorobenzene (PNCB), o-nitrochlorobenzene (ONCB) and m-nitrochlorobenzene (MNCB), are manufactured by a single US producer, Solutia Inc., at a single manufacturing site in an essentially closed, continuous process. Only a few employees are involved in the manufacturing operations and have minimal potential for skin or airborne exposure, which occurs chiefly during material transfer operations.

All three Chloronitrobenzene isomers, PNCB, ONCB and MNCB are known to produce methemoglobinemia in human and animals (Linch, 1974) and are considered hazardous after dermal contact. Addition of the nitro group in the *para* position relative to the chlorine group on the benzene molecule results in the formation of the most toxic of the three isomers. Potency of response in both humans and animals is equivalent to para> meta>>ortho (Watanabe et al, 1976; Davydova, 1967). To minimize the potential for adverse health effects due to methemoglobinemia resulting from occupational exposure via inhalation or skin absorption, a TLV ® of 0.1 ppm (~0.64 mg/m^3) has been established for PNCB (ACGIH, 2001). While comparative toxicity and occupational experience indicate that MNCB and ONCB produce less toxicity and a lower risk of methemoglobinemia, an internal Solutia Inc. occupational standard of 1 mg/m^3 has also been set for these chemicals. In all cases, specific manufacturing procedures and practices have been established to minimize occupational exposure potential.

PNCB and ONCB are important chemical intermediates that serve as basic building blocks for the manufacture of numerous industrial chemicals. For example, PNCB is utilized via chemical reaction to make industrial chemicals that are ultimately used in the preparation of dyes and pigments, pesticides, and animal feed ingredients. ONCB is converted in similar fashion to dyes and pigments, polymer additives, veterinary pharmaceuticals and water-treatment chemicals. MNCB has limited use as a chemical intermediate.

Chloronitrobenzenes are sold to a limited number of customers at a few processing sites for the express purpose of full chemical conversion into other industrial chemicals. There are no known or suspected consumer exposures to these chemicals resulting from TSCA-related activities, as they are fully consumed as chemical intermediates. Loss to the atmosphere or from non-POTW aqueous streams during manufacturing or processing is minimal. Hence, very limited occupational or environmental exposure is expected to occur.

II. CATEGORY JUSTIFICATION

For purposes of the HPV Challenge Program, EPA has provided guidance as to the definition and justifications to be used in selection of a chemical Category (US EPA, 1999c). The definition states that a chemical Category should be "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". Solutia Inc. has opted to form the Chloronitrobenzene Category with this guidance in mind.

Common Structure

The three chemicals selected for inclusion in this category are isomeric forms of the same base chemical, nitrobenzene. Hence, they are of common structure.

Common Functional Groups

Each of these nitrobenzene compounds are aromatic hydrocarbons for which one benzene ring hydrogen has been replaced by a nitro (NO2) radical and one benzene ring hydrogen further replaced with a chloro (Cl) group; the position (either *ortho* to, *meta* to, or *para* to the chloro grouping) of the ring placement of the nitro grouping is the only structural difference between these three isomers. For the most part, these compounds are similar in chemical properties, as well as in their pharmacological or toxicological effects. As such these effects are modified to a greater or lesser degree by the location of the substituent radicals (Beard and Noe, 1982; Davydova, 1967; Watanabe et al, 1976).

Similar or even Identical Properties or Hazards

Physicochemical properties of these three isomeric forms of the same chemical are quite similar. Their physical form is crystalline and their molecular weights and specific gravity are identical. Other parameters are similar, but not identical. A summary of available physicochemical data can be found in Table 4.

Environmental Fate data are summarized in Table 5. A large body of published information exists in this data category. Whether measured or estimated, there appears close agreement in each of the HPV Endpoints recorded for each of the chemicals in this category.

Comparative aquatic toxicity of the members of this Category can be found in Table 6. As shown, a similar degree of toxicity has been observed across the multiple test species included in this dataset.

Tables 7 - 10 summarize the comparative mammalian toxicity of these chemicals. It is well recognized that all three of these chemicals possess a similar mode of action. Their toxicity is characterized by a common and outstanding property, i.e., their ability to form methemoglobin (Beard and Noe, 1982) in both humans and animals. Comparative investigations have established the order of potency to be: para isomer > meta isomer >> ortho isomer (Watanabe et al, 1976; Davydova, 1967). However, there are marked species differences in susceptibility to methemoglobinemia with humans being decidedly more affected than rodent species. Thus, results of acute toxicity studies in rodents are not considered fully representative of the high acute toxicity to humans that can be elicited by these chemicals. On the basis of past human experience, where dermal contact or inhalation exposures resulted in incidences of methemoglobinemia, unusually diligent care has been taken to insure proper handling of both chemicals (each treated equally) during manufacture, shipment, disposal and use.

Thus, similarities in the chemical structure, biological mode of action and the extensive comparative data sets presented support use of a Category approach for these chemicals.

III. TEST PLAN RATIONALE

The information obtained and included to support this Test Plan have come from either 1) internal studies conducted by/or for Solutia Inc. (or its predecessor Monsanto Co.), 2) have been extracted from the scientific literature either as primary references or as found in well-accepted, peer-reviewed reference books, or 3) were estimated using environmental models accepted by the US EPA (1999b) for such purposes. This initial assessment includes information on physicochemical properties, environmental fate, and human and environmental effects associated with each member of this Category. The data used to support this program include those endpoints identified by the US EPA (1998); key studies have been identified for each data Endpoint and summarized in Robust Summary form and included in Section VII of this dossier.

All studies were reviewed and assessed for reliability according to standards specified by Klimisch *et al* (1997), as recommended by the US EPA (1999a). The following criteria were used for codification:

1. Reliable without Restriction - Includes studies which comply with US EPA and/or OECD-accepted testing guidelines, which were conducted using Good Laboratory Practices (GLPs) and for which test parameters are complete and well documented,

2. Reliable with Restriction – Includes studies which were conducted according to national/international testing guidance and are well documented. May include studies conducted prior to establishment of testing standards or GLPs but meet the test parameters and data documentation of subsequent guidance; also includes studies with test parameters which are well documented and scientifically valid but vary slightly from current testing guidance. Also included were physical-chemical property data obtained from reference handbooks as well as environmental endpoint values obtained from an accepted method of estimation (i.e. EPIWIN).

3. Not Reliable – Includes studies in which there are interferences in either the study design or results that provide scientific uncertainty or where documentation is insufficient.

4. Not Assignable – This designation is used in this dossier for studies which appear scientifically valid but for which insufficient information is available to adequately judge robustness.

Those studies receiving a Klimisch rating of 1 or 2 are considered adequate to support data assessment needs in this Dossier. Those key studies selected for

inclusion are considered typical of the Endpoint responses observed in other studies of a similar nature and design, which were identified during our search of the literature; additional references can be found in the current ECB IUCLID dossiers for o-Chloronitrobenzene (2000), m-Chloronitrobenzene (2000) and p-Chloronitrobenzene (2000), as referenced below.

IV. TEST PLAN SUMMARIES AND CONCLUSIONS

The referenced available data for each Category member have been placed in an Endpoint-specific matrix and summarized individually in Table 1 (ONCB), Table 2 (MNCB) and Table 3 (PNCB). Substantial data exists for each chemical to evaluate its potential hazards in this screening level assessment. Where an HPV Endpoint has been untested, the need for testing has been assessed (1) with the understanding that these chemicals behave in a similar and/or predictable manner, and (2) by interpolation (i.e. Read-Across technique) between data from other key studies already available. Thus, we have used preexisting data, where possible, to support our assessment of potential hazards of the chemicals in this Category and avoid the unnecessary testing of additional laboratory animals.

Conclusion: All HPV Endpoints have been satisfied for the three Chloronitrobenzene isomers with data from studies that were either well documented, used OECD guideline methods and conducted in accord with GLPs, or were estimated from acceptable estimation modeling programs. Use of the "Read Across" technique was employed sparingly to support a limited number of endpoints. Hence, no further testing for any of the HPV endpoints is deemed necessary (Tables 1, 2 and 3).

Physical-chemical property values - Melting Point and Boiling Point values for all three Chloronitrobenzenes were obtained from reputable references and cited as an Accepted or Peer Reviewed value in their respective Hazardous Substances Data Banks (2002). Measured values were found for Vapor Pressures and Partition Coefficients from reputable studies, and which were also cited in accepted peer reviewed documents. The Water Solubility of each Chloronitrobenzene was estimated using an accepted methodology. Thus, in all cases these values were given a classification of "2-Reliable with restrictions".

Environmental Fate values describing Transport (Fugacity) for ONCB, MNCB and PNCB were obtained using a computer estimation –modeling program (EPIWIN, 2002) recommended by EPA and classified as "2-Reliable with restrictions". Photodegradation and Biodegradation data for each of the three Chloronitrobenzene isomers were characterized in well-documented studies, the latter conducted

according to ASTM/EPA guidelines that since have been codified and are similar to OECD test #301 guidance. These studies thus are classified as "2-Reliable with restrictions". No Stability in Water (hydrolysis) data were found for any of the three Chloronitrobenzenes. Further, water solubility values could not be calculated using EPIWIN, as these chemicals are know to be resistant to hydrolysis.

Ecotoxicity – Acute Fish, Invertebrate and Plant Toxicity Endpoints for PNCB and ONCB have been fulfilled with studies, most of which were conducted according to US EPA test guidance consistent with OECD test guidelines. All studies were well documented and were designated "2-Reliable with restrictions". An Acute Fish Toxicity study, also designated as "2-Reliable with restrictions", has been included for MNCB. The Acute Invertebrate and Plant Toxicity Endpoints for MNCB are fulfilled using the 'Read Across" method of data evaluation, as no fully reliable studies were found in these two areas. Utility of this methodology is strengthened by comparative use of estimation modeling data as well as literature information deemed limited ("4-Not Assignable") in documentation, but useful for supportive purposes.

Mammalian Toxicity Endpoints, including Acute Toxicity, Repeated Dose Toxicity, Ames Mutagenicity, Chromosomal Aberration Testing and Reproductive Toxicity for both PNCB and ONCB have been fulfilled by way of tests that either conformed directly to OECD test guidance or followed test designs similar to OECD guidance. Thus, they have been designated either "1-Reliable without restriction" or "2-Reliable with restrictions".

An Acute Toxicity study, an Ames test and a Cytogenetics study have been conducted with MNCB and fulfill these Endpoint requirements for this isomer; each of these studies has been designated as either "1-Reliable without restriction" or "2-Reliable with restrictions". No Repeated Dose Toxicity (of sufficient reliability) or Reproductive Toxicity studies have been identified for MNCB. Thus, these Endpoints have been filled using the "Read Across" technique for data assessment, since both the ortho and para isomers have been extensively evaluated for these Endpoints.

Based on the conclusions as outlined above on HPV Endpoint assessment, following is a tabular depiction of data availability and testing recommendations for ortho-Chloronitrobenzene (ONCB) (Table 1), meta-Chloronitrobenzene (MNCB) (Table 2) and para-Chloronitrobenzene (PNCB) (Table 3).

Table 1. Test Plan Matrix for ortho-Chloronitrobenzene (ONCB)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept-Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	N	Y	N
Boiling Point	Y	N	N	R	N	Y	N
Vapor Pressure	Y	N	N	R	N	Y	N
Partition Coefficient	Y	N	N	R	N	Y	N
Water Solubility	Y	N	N	R	Y	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	Y	N	Y	N
Stability in Water	N	-	-	-	-	Y	N
Biodegradation	Y	N	N	Y	N	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	N	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	N	N	Y	N	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	N	Y	N	Y	N
Acute Toxicity to Aquatic Plants	Y	N	N	Y	N	Y	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	N	N	Y	N	Y	N
Repeated Dose Toxicity	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Mutation (Ames)	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	N	Y	N	N	Y	N
Reproductive Toxicity	Y	N	Y	N	N	Y	N
Developmental Toxicity	Y	Y	Y	Y	N	Y	N

Y = Yes; N = No; R = Reputable Reference; - = Not applicable

Table 2. Test Plan Matrix for meta-Chloronitrobenzene (MNCB)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept-Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	N	Y	N
Boiling Point	Y	N	N	R	N	Y	N
Vapor Pressure	Y	N	N	R	N	Y	N
Partition Coefficient	Y	Y	Y	R	N	Y	N
Water Solubility	Y	N	N	R	Y	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	Y	N	Y	N
Stability in Water	N	-	-	-	-	Y	N
Biodegradation	Y	N	N	Y	N	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	N	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	N	N	Y	N	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	N	Y	Y	C	N
Acute Toxicity to Aquatic Plants	Y	N	N	Y	Y	C	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	Y	Y	Y	N	Y	N
Repeated Dose Toxicity	Y	N	N	Y	N	C	N
Genetic Toxicity – Mutation (Ames)	Y	N	N	Y	N	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	N	Y	N	N	Y	N
Reproductive Toxicity	N	-	-	-	-	C	N
Developmental Toxicity	N	-	-	-	-	-	-

Y = Yes; N = No; R = Reputable Reference; ; - = Not applicable
C = Read-across from available data on ONCB & PNCB

Table 3. Test Plan Matrix for para-Chloronitrobenzene (PNCB)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept-Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	N	Y	N
Boiling Point	Y	N	N	R	N	Y	N
Vapor Pressure	Y	N	N	R	N	Y	N
Partition Coefficient	Y	N	N	R	N	Y	N
Water Solubility	Y	N	N	R	Y	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	Y	N	Y	N
Stability in Water	N	-	-	-	-	Y	N
Biodegradation	Y	N	N	Y	N	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	N	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	N	Y	Y	N	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	Y	Y	N	Y	N
Acute Toxicity to Aquatic Plants	Y	N	N	Y	N	Y	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	N	N	Y	N	Y	N
Repeated Dose Toxicity	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Mutation (Ames)	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	Y	Y	Y	N	Y	N
Reproductive Toxicity	Y	Y	Y	Y	N	Y	N
Developmental Toxicity	Y	Y	Y	Y	N	Y	N

Y = Yes; N = No; R = Reputable Reference; - = Not applicable

V. Data Set Summaries and Evaluations

The key studies used in this assessment to fulfill the HPV requirements for ONCB, MNCB and PNCB have been placed in an Endpoint-specific matrix, and further discussed below. As a number of studies supporting many of these Endpoints exist for each Chloronitrobenzene, key studies were selected based on their representative presentation of data characterization as well as their reliability. Robust Summaries for each study referenced can be found in Section VII of this dossier.

A. Chemical/Physical Properties

A large number of studies are available summarizing the **Physical-Chemical** properties associated with these Chloronitrobenzenes. They can be found in ECB IUCLID Dossiers for o-Chloronitrobenzene (2000), m-Chloronitrobenzene (2000) and p-Chloronitrobenzene (2000). Table 4 contains those values that are considered to best depict the consensus of results found in most key sources used to define the characteristics of each of these Chloronitrobenzenes. They have been obtained from reputable reference books or measured values and cited in peer-reviewed data sources; thus, they are considered "2-Reliable with restrictions". A Robust Summary has been prepared for each of the references included in Table 4.

In summary, ONCB, MNCB, and PNCB are solid entities at room temperature and possess low vapor pressures. They have a moderate partition coefficient and are moderately soluble in water.

Conclusion: Sufficient data exists to fully characterize the Physical-Chemical properties of each of these Chloronitrobenzenes. All HPV data requirements for this Endpoint have been met and no further data collection is planned.

Table 4. Selected Physical Properties of Chloronitrobenzenes

Chemical	Boiling Pt. (^{o}C.)	Melting Pt. (^{o} C.)	Vapor Pressure (hPa @ 25 ^{o}C)	Water Solubility (mg/L)	Partition Coeffient (Log Kow)
o-Chloronitrobenzene CAS No. 88-73-3	245.7	32.5	0.0575 @ 20 oC	307	2.24
m-Chloronitrobenzene CAS No. 121-3	236	46	0.129	256	2.49
p-Chloronitrobenzene CAS No. 100-00-5	242	83.4	0.1253	154	2.39

D. Environmental Fate and Biodegradation

Semi-Continuous Activated Sludge (SCAS) Biodegradability studies have been conducted to assess the biodegradation potential of ONCB and PNCB; they have been summarized in the Robust Summary section of this Dossier and cited in Table 5 below. While each study was conducted prior to inception of standardized international guidelines for **Biodegradability** testing and GLPs, they followed similar standards for conduct subsequently codified into OECD guideline 301 and GLP documentation. Thus, they are each considered "2-Reliable with restrictions". An anaerobic bacterial assay with MNCB was selected to fulfill this HPV data requirement as it was well documented and thus also considered "2-Reliable with restrictions". Supplemental studies summarized in Section VII for each compound confirm the conclusion that Chloronitrobenzenes undergo slow biodegradation in non-adapted soil.

A single, comparative study of the photochemical reactions associated with each of the three Chloronitrobenzenes has been summarized in the Robust Summary section of this dossier. It has been classified as "2-Reliable with restrictions", as it provides useful information, appears well conducted, but did not conform to codified OECD guidelines. Comparative values have been included in Table 5. AOPWIN modeling for this **Photodegradation** Endpoint has also been included for comparative purposes.

We have incorporated the use of an estimation model (EPIWIN, 2002) for determination of Transport Between Environmental Compartments (**Fugacity),** for all three Chloronitrobenzenes**.** A Fugacity Level III model was used in each case, and employed measured values, where possible, as recommended by the US EPA. Thus, the estimations derived from each of these models have been classified as "2-Reliable with restrictions". These estimates have also been included in Table 5 and are cited in the Robust Summary section of this Dossier; data entries used in the Level III fugacity model have been included in the Robust Summaries for validation of output.

No values have been identified to define the **Stability in Water** (hydrolysis) of any of these Chloronitrobenzenes. Further no such values could be calculated using EPIWIN (2002) as each chemical has only aromatic nitro and aromatic chloro functional groups, both of which are listed in Lyman et al. (1990) as Generally Resistant to Hydrolysis. Thus, "[t]esting for Stability in Water is not needed for substances generally recognized to have molecular structures or possess only functional groups that are generally known to be resistant to hydrolysis" (OECD, 2002).

Conclusion: Sufficient information exists to characterize the Environmental Fate and Biodegradation of each of these Chloronitrobenzenes. Where experimental data do not exist, use of an estimation model (EPIWIN) recommended by EPA provided necessary information or the rationale lack of need for testing has already been recognized. Thus, all HPV data requirements for this Endpoint are met and no further data collection is planned.

Table 5. Comparison of Environmental Fate Endpoints for Category Members

Chemical	Biodegradation Rate	Stability in Water	Photodegradation (% Disappeared-5 Hr Irradiation)	Fugacity (%)
o-Chloronitrobenzene CAS No. 88-73-3	11-48 % Primary Degrad.(SCAS)	n.d.	66	Air- 6.5 Water- 33.5 Soil- 59.8 Sediment-0.16
m-Chloronitrobenzene CAS No. 121-73-3	50% (anaerobic sediment)	n.d.	89	Air- 8.0 Water- 28.8 Soil- 63.0 Sediment-0.19
p-Chloronitrobenzene CAS No. 100-00-5	31-66% Primary Degrad. (SCAS)	n.d.	96	Air- 9.5 Water- 28.5 Soil- 61.8 Sediment- 0.17

nd. = no data available

To summarize the Environmental fate of these Chloronitrobenzenes, based on Fugacity modeling the members of this Category are expected to be found primarily in the soil and water as main environmental target compartments. None of these chemicals is readily hydrolysable in the environment. They can be abiotically reduced in the presence of natural electron transport mediators and under reducing conditions, but are not Readily Biodegradable. Under conditions of domestic waste treatment, considerable biodegradation is apparent. Estimated Koc values suggest the Chloronitrobenzenes possess moderate mobility in soils (EPIWIN, 2002); slow volatilization is expected to occur, based on their vapor pressures. These chemicals are expected to exist primarily in the vapor phase in the atmosphere where they will degrade slowly by reaction with photochemically producing hydroxyl radicals.

E. Aquatic Toxicity

Several references to acute fish, invertebrate and algal toxicity can be found in the ECB IUCLID documents for ONCB (2000), MNCB (2000) and PNCB (2000). Data presented in Table 6, and summarized in the Robust Summary section VII, depict the level of toxicity generally observed for these Endpoints within the overall dataset. All of the studies selected to fulfill the Acute Fish, Acute Invertebrate and Acute Plant Toxicity Endpoints for ONCB and PNCB were either conducted according to US EPA test guidance (ASTM/EPA) consistent with international guidance or published in a peer-reviewed journal possessing sufficient documentation. Thus, they are considered "2-Reliable with restrictions". Similarly, a well-documented Acute Fish toxicity study with MNCB, which followed US EPA/ASTM guidance, is also considered "2-Reliable with restrictions". Two literature articles were found summarizing acute toxicity effects of

MNCB in Daphnia and algae. Both purportedly were conducted following OECD or Dutch National testing guidance. Additionally, both articles provided a comparative assessment of all three Chloronitrobenzene isomers considered in this Category. However, neither article provides sufficient detail nor individual data documentation to be assigned a reliability code other than "4- Not assignable" for HPV purposes. A Robust Summary has been completed for each study and included in the Robust Summary Section of all three isomers in Section VII of this Dossier. Additionally, we have conducted estimation modeling for a 48-hr Daphnid LC50 and a 96-hr Algae EC50 for all three Chloronitrobenzene isomers using ECOSAR (US EPA, 2002), including MNCB. These estimates have been included in Table 6 and further summarized in Robust Summary form in Section VII. In summary, the empirical data derived from testing and the estimations derived from modeling, support a similar degree of comparative acute aquatic toxicity of all three Chloronitrobenzene isomers to these three aquatic species. Thus, it is reasonable and justifiable to use the "Read Across" technique for fulfilling both the Acute Invertebrate and Acute Aquatic Plant Toxicity Endpoints for MNCB from empirically derived data available for both ONCB and PNCB.

Conclusion: Sufficient data exists to fully characterize the Acute Aquatic Toxicity properties of each of these Chloronitrobenzenes. All HPV data requirements for this Endpoint have been met with empirical data or through limited and scientifically justified "Read Across" methods such that no further data collection is required for these materials.

Table 6. Comparison of Aquatic toxicity parameters for category members

Chemical	Fish LC 50 (mg/L) (96-hr)	Invertebrate (Daphnia) LC50 (mg/L) (48-hr)	Algae EC50 (mg/L) (48-hr)
o-Chloronitrobenzene CAS No. 88-73-3	30.3 (Guppy)	41.0	34.0 (biomass)
m-Chloronitrobenzene CAS No. 121-73-3	18.8 (F. minnow)	47.7 (estim.)	30.6 (estim.)
p-Chloronitrobenzene CAS No. 100-00-5	6.0 (R. trout)	10.0	8.0 (biomass)

D. Mammalian Toxicity

1.0 Acute Toxicity

Key acute toxicity studies by the oral exposure route were chosen from a number of other acute reports; these results represent acute toxicity values identified from reliable sources. It should be noted that acute toxicity studies with most laboratory animals are not considered sufficiently predictive of the acute hazards of these nitroanilines to humans, due to the resistance observed in lab animals to development of methemoglobinemia. All studies included in Table 7 were conducted specifically or in general agreement with OECD acute toxicity testing guidance and are considered either "1-Reliable without restriction" or "2-Reliable with restrictions". Other acute toxicity study results are cited in the ECB IUCLID dossiers for ONCB (2000), MNCB (2000) and PNCB (2000).

Table 7. Acute Mammalian Toxicity for Category members

Chemical	Rat Oral LD50 (mg/kg)
o-Chloronitrobenzene CAS NO. 88-73-3	560
m-Chloronitrobenzene CAS No. 121-73-3	400
p-Chloronitrobenzene CAS No. 100-00-5	530

Conclusion: Sufficient data from well-documented studies (Acute Oral Toxicity) exist to meet the Acute Toxicity data set requirements for all members of this Category. Hence, no further acute toxicity testing is planned.

2.0 Repeated Dose Toxicity

PNCB and ONCB have been extensively evaluated in Repeated Dosing studies of various durations and by different exposure routes (ECB IUCLID - PNCB, 2000; ECB IUCLID – ONCB, 2000). Studies conducted in rats for 13 weeks by the inhalation exposure route with ONCB and PNCB, each consistent with OECD Test Guideline 413, have been selected to fulfill the requirements for this HPV Endpoint. Each of those studies is summarized in Table 8, is considered "1-Reliable without restriction" and has been included in the Robust Summary section of this dossier. Additional Repeated Dose rat inhalation studies of a shorter duration (4-weeks), have been included as Supplemental information in Table 8 and summarized in the Robust Summary section of this dossier, as they are useful for comparative purposes. Additionally, it should be noted that other Repeated Oral Dose studies with PNCB are available and have previously been submitted to EPA and are cited in the ECB IUCLID – PNCB (2000). These studies include: a

chronic/carcinogenic oral rat study (Nair et al, 1989), a 13-week oral toxicity study in rats (Solutia, 1979).

No adequately reported Repeated Dose studies were found for MNCB after an extensive literature search as well as review of its ECB IUCLID (2000) document. However, the summary of a series of studies comparing MNCB repeated dose toxicity with that of PNCB and ONCB was found (Davydova, 1967). It has been included in this discussion as it provides some useful Supplemental information. Due to its inclusion as only summary data, it has been assigned a Reliability classification of "4-Not Assignable". While included in the Robust Summary section of this dossier, it has not been included in Table 8.

Conclusion: The Repeated Dose HPV Endpoint for both PNCB and ONCB are complete with selection of a 13-week inhalation study in rats for each chemical, as each meets OECD Test Guideline 413; thus, no further testing is needed.

It is scientifically justifiable to consider completion of the Repeated Dose HPV Endpoint for MNCB through use of the "Read Across" technique for data assessment, based on 1) similarity of structure, i.e. it is one of three nitrobenzene isomers considered in this dossier, 2) substantive and fully adequate testing for this Endpoint already exists for the other two isomeric forms, PNCB and ONCB, 3) there is a known, identical mode of action associated with all three isomers (methemoglobinemia) and 4) a consistent pattern of repeated dose toxicity has been established among the three isomers. Clinical observations, serum chemistry changes, organ weight differences and histopathological findings associated with PNCB and ONCB were related to methemoglobin formation and compensatory processes that occurred as a result. The single Supplemental study found in the literature with MNCB characterized its repeated dose toxicity as fully comparable with that seen with PNCB and ONCB. However, the degree of potency of MNCB was characterized as closer to the more toxic isomer, PNCB, rather than ONCB, the lesser toxic isomer.

Conclusion: "Read Across" methodology, based on the use of reliable data from PNCB and ONCB, is scientifically justified to adequately characterize the Repeated Dose hazards associated with MNCB. Thus, the requirements for the Repeated Dose HPV Endpoint for MNCB are complete and no further, unnecessary animal testing is warranted.

Table 8. Repeated Dose Toxicity Studies with Category Members

Chemical	Study Type	Dosages	Histopathology	Hematology/Clinical Findings
o-Chloronitro-benzene CAS NO. 88-73-3	13-Week Rat Inhalation 10M/10F/group F344 rats	18 ppm	Respir. Epithel.-hyperplasia Liver-basophilia Spleen-congestion Kidney-hemosiderosis Kidney, Liver,Spleen Wt	MET, RETIC, SDH, LB,ALT, AP, B acids HCT, HGB, RBC, PLAT
		9 ppm	Respir. Epithel.-hyperplasia Liver-basophilia Kidney-hemosiderosis Kidney, Liver Wt	MET, RETIC, SDH, LB,ALT, AP, B acids HCT, HGB, RBC, PLAT, MCHC/MCH(F)
		4.5 ppm	Respir. Epithel.-hyperplasia Liver-basophilia Kidney-hemosiderosis Spleen Wt	MET, SDH, ALB,ALT, B acids HCT, HGB, RBC
		2.3 ppm	Respir. Epithel.-hyperplasia Liver Wt	MET, SDH, ALB,ALT, B acids; HCT
		1.1 ppm	Respir. Epithel.-hyperplasia	MET
o-Chloronitro-benzene CAS NO. 88-73-3	4-Week Rat Inhalation 15M/15F/group S-D Rats	60 mg/m3 (~9.3 ppm)	Spleen-Extramed. Hematopoiesis & hemosiderosis Liver, Kidney, & Spleen Wt	MET, RET HCT, HGB, RBC
		30 mg/m3 (~4.6 ppm)	Spleen-Extramed. Hematopoiesis & hemosiderosis Liver, Kidney, & Spleen Wt	MET HCT (F), HGB (F), RBC (F) -
		10 mg/m3 (~1.5 ppm)	Liver Wt (M)	
m-Chloronitro-benzene CAS No.121-73-3		No Data		

p-Chloronitro-benzene (PNCB) CAS No.100-00-5	13-Week Rat Inhalation 10M/10F/group F344 rats	24 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis/fibrosis - hyperplasia Testes - atrophy Liver, Spleen, Heart, Thymus, Testes weights	MET, RET, MCH, n-RBC, SDH, B acids HCT, RBC, HGB, AP, GLOB, ALT, TPROT
		12 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis/fibrosis - hyperplasia Liver, Spleen, Heart weights	MET, RET, n-RBC, SDH, B acids HCT, RBC, HGB, AP, GLOB, ALT, TPROT
		6 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis/fibrosis - hyperplasia Liver, Spleen weights	MET, RET, n-RBC, SDH (F), B acids (M) HCT, RBC, AP, GLOB, ALT, TPROT
		3 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis Liver, Spleen weights	MET, RET, n-RBC, B acids (M) HCT, HGB, RBC, ALT (M)
		1.5 ppm	Renal-hyaline droplets (M only) Spleen -hemosiderosis	MET, RET, n-RBC HCT, HGB, RBC, ALT (M)
p-Chloronitro-benzene (PNCB) CAS No.100-00-5	4-Week Rat Inhalation 15M & 15F/group S-D rats	45 mg/m3 (~ 7 ppm)	Spleen-congestion & hemosiderosis & Extramedullary hematopoiesis Liver & Spleen weight	MET HCT, HGB, RBC

		15 mg/m3 (~ 2.3 ppm)	Spleen – hemosiderosis Liver weight (F)	MET HCT, HGB, RBC
		5 mg/m3 (~ 0.8 ppm)	Spleen - hemosiderosis	HCT, HGB, RBC

3. Mutagenicity and Chromosomal Aberrations

Ames Test

For each of the three Chloronitrobenzene isomers, a key point mutation study has been selected to fulfill this HPV Endpoint. Both the ONCB and PNCB studies were conducted according to GLPs and conformed to OECD Test Guideline 471 and thus are considered "1-Reliable without restriction". The study with MNCB was well documented but conducted prior to OECD Test Guideline codification and thus is considered "2-Reliable with restrictions". Each study has been cited in Table 9 as well as extensively summarized in the Robust Study section of this Dossier. Additional Ames test assays are reported in the ECB IUCLID for ONCB (2000), MNCB (2000), and PNCB (2000).

Weak positive responses were seen in Salmonella with ONCB and PNCB but not MNCB. Both ONCB and PNCB have been consistently inactive (negative) in *in vitro* assays using mammalian cell lines, including the CHO/HGPRT assay (Solutia 1982a, 1983a), the UDS Rat Hepatocyte Culture assay (Solutia 1983b, 1984) and a rat hepatocyte DNA repair assay with PNCB (Solutia, 1982b). PNCB was positive only with metabolic activation in the Mouse Lymphoma assay (Solutia, 1981). Neither PNCB nor ONCB induced sex-linked recessive lethal germ cell mutations in an *in vivo*, secondary tier mutation assay (NTP, 1993).

Conclusion: The Ames Test Category Endpoint for each of the Chloronitrobenzenes has been met and no further testing should be considered for the gene point mutation endpoint for this chemical.

Table 9. Genetic Toxicity of Category Members

Chemical	Ames Test- TA98, 100, 1535, 1537 +/- activation	Cytogenetics In Vitro (CHO Cells)	Cytogenetics In Vivo
o-Chloronitro-benzene CAS NO. 88-73-3	Positive – TA100 w S-9 Negative – TA100 w/o S-9 Negative -. w & w/o S-9. TA98, TA1535, TA1537	Weak Positive- w S-9 Negative – w/o S-9	 n.d.
m-Chloronitro-benzene CAS No. 121-73-3	Negative – TA100, TA98, TA1535, TA1537, TA1538 w and w/o S-9	Negative - w & w/o S-9	 n.d.
p-Chloronitro-benzene CAS No, 100-00-5	Positive – TA1535 w/o S-9 Ambiguous- TA1535 w S-9 Negative – TA98, TA1537, TA100 w and w/o S-9	Weak Positive – w & w/o S-9	Negative

n.d. = no data

Chromosomal Aberrations -

Three *in vitro* CHO cell chromosomal aberration studies sponsored by the US NTP program, each with a different Chloronitrobenzene isomer, have been conducted following a study design similar to, but not identical with, OECD Test guideline 473. Each study was well documented and followed GLPs and thus is considered to be "2-Reliable with restrictions". These studies have been used to fulfill this HPV Endpoint for ONCB and MNCB. However, while the CHO cell study could be used to support this Endpoint for PNCB, a secondary tier, *in vivo* Chromosomal Aberration Test (classified "2-Reliable with restrictions") has been chosen as the key HPV study for this chemical.

Conclusion: On the basis of reliable in vitro (ONCB and MNCB) and in vivo (PNCB) Chromosomal Aberration Assays available for each of these Chloronitrobenzenes, no additional testing is needed to fulfill this HPV Endpoint.

4. Reproductive and Developmental Toxicity

PNCB, the most toxic chemical in this Chloronitrobenzene group, has undergone extensive testing for developmental toxicity in two species (rat and rabbit) and has been evaluated both in a rat Two-Generation Reproduction study and a mouse Continuous Breeding study. Each of these studies have been assessed as "1-Valid without restriction" as they fully met OECD testing (or standardized methodology as in the case of the Continuous Breeding study) and GLP guidance. The Two

Generation Rat Reproduction study has been selected as the key study to fulfill this HPV Endpoint for PNCB as its design is considered more conventional than the Continuous Breeding study. The developmental toxicity studies are included as Supplemental information. Each of these adequately conducted studies has been summarized in Table 10 and Robust Summaries developed.

ONCB has been evaluated in a comparative (to PNCB) rat teratology study. This study has also been evaluated as being "1-Valid without restriction" and has been summarized in Table 10. Additionally, it has been tested in a mouse Continuous Breeding study, as has PNCB. As the Continuous Breeding study was conducted in accord with standardized testing methodology for this reproduction study and under GLPs, it has been classified as "1-Reliable without restriction" and fulfills the Reproductive Toxicity HPV Endpoint for ONCB. Robust Summaries for each study can be found in Section VII of this Dossier.

To summarize the available information on these two Chloronitrobenzene isomers, ONCB was judged "not to be a reproductive toxicant, even in the presence of systemic toxicity in Swiss CD-1 mice" (NTP, 1993). PNCB produced no effects on reproductive toxicity parameters through 2 generations in rats up to a level (5 mg/kg/d) known to produce significant systemic toxicity (Nair et al, 1989). Significant and progressive deficits in infertility in the FO generation and reduced weight gains in F1 and F2 pups were seen in mice during the Continuous Breeding study and may have been related to methemoglobin-related hypoxia associated with cyanosis observed at PNCB test levels. Developmental toxicity was seen only at the highest dose tested in rats with PNCB, and thus was judged to not to have a primary effect on fetal development. ONCB produced no developmental toxicity when evaluated in rats even at maternally toxic levels.

No Reproductive Toxicity or Developmental Toxicity studies have been identified with MNCB. However, we believe sufficient data exists in this Category to obviate the need for further evaluation of MNCB, based on the similarity of mammalian toxicity of this group of Chloronitrobenzene isomers and through use of the corresponding reproductive toxicity data available on both PNCB and ONCB. A "Read Across" approach, using the PNCB and ONCB reproductive studies in rats and mice, has been used to fulfill the Reproductive Toxicity HPV Endpoint for MNCB. As there are differences noted in potency and effects seen between PNCB (greater toxicity) and ONCB (lesser toxicity)(see below), we believe it appropriate to associate similarity of effects projected with MNCB with those of PNCB. This provides both a more conservative approach to assignment of effects as well as the most scientifically justifiable, as human experience and repeated dose testing in animals support closer analogy of response between MNCB and PNCB than between MNCB and ONCB.

Thus, we conclude that use of all available data in the Category approach, along with key studies with ONCB and PNCB, allows this HPV Endpoint to be completed without further unnecessary testing of MNCB.

Table 10. Summary of Developmental Toxicity and Reproduction Studies with Category Members

Chemical	Study Type/Species	Dosage	Observations	Conclusion
o-Chloronitro-benzene (ONCB) CAS NO. 88-73-3	Rat Teratology – Gavage 25 /group	150 mg/kg	Maternal Toxicity: 6/25 early deaths	no further investigation
		100 mg/kg	Maternal Toxicity: Body wt gain Food consump. 1 death; No terata, embryotox or fetotox	NOEL for Embryotoxicity, Fetotoxicity, Teratogenicity
		75 mg/kg	Maternal tox; Food consump. 1 death	
		25 mg/kg	No findings	NOEL for Maternal toxicity
o-Chloronitro-benzene (ONCB) CAS NO. 88-73-3	Mouse Continuous Breeding	160 mg/kg	Methem in FO & F1 FO (M/F) spleen wts F1(m) spleen and liver wts ; sem. Vesic.Wt F1 (Final litter) M/F pup wt.	NOEL – fertility Indices
		80 mg/kg	FO (M/F) spleen wts F1 (Final litter) M/F pup wt.	
		40 mg/kg	F1 (Final litter) female pup wt.	
m-Chloronitro-benzene (MNCB) CAS NO. 121-73-3	No studies found			
p-Chloronitro-benzene (PNCB)	Rat Teratology – Gavage 25/group	45 mg/kg	Maternal toxicity: Body wt. Gain Spleen wt. Embryotoxicity: Resorptions Fetotoxicity: Fetal wts.	

CAS No. 100-00-5		15 mg/kg 5 mg/kg	Terata: skeletal Maternal toxicity: Body wt. Gain Spleen wt. No terata, embryo- or fetotoxicity No findings	NOEL for teratogenicity, fetotoxicity and embryotoxicity Maternal toxicity NOEL
p-Chloronitro-benzene (PNCB) CAS No. 100-00-5	Rabbit Teratology-Gavage 18/group	125 mg/kg 75 mg/kg 25 mg/kg	Maternal Toxicity: Deaths (7/18) Physical changes Maternal toxicity: Physical changes No findings	NOEL for Terata, fetotoxicity, and embryotoxicity NOAEL for Maternal Toxicity Unequivocal NOEL for Maternal Toxicity
p-Chloronitro-benzene (PNCB) CAS No. 100-00-5	Two-generation Rat Gavage Reproduction Study 15 males/30 females per group in F0 and F1 generations	5 mg/kg 0.7 mg/kg 0.1 mg/kg	Maternal toxicity: Histopathology consistent with methemoglobinemia F0/F1: all mating indices judged normal No findings No findings	NOEL for all reproductive endpoints NOEL: Maternal toxicity
p-Chloronitro-benzene (PNCB) CAS No. 100-00-5	Mouse Continuous Breeding	250 mg/kg 125 mg/kg	Most animals visibly cyanotic FO-Fertility (after 1st litter) F1-spleen and liver wt ; estrus cycle F1 & F2 pup wt F2 pup survival and wts FO-Fertility (after 1st litter) F1 & F2 pup wt	

		62.5 mg/kg	FO-Fertility (after 1st litter) F1 male pup wt	

In summary, as seen previously in sections dealing with acute and repeated dose testing for mammalian toxicity endpoints, PNCB has proven to produce the more significant comparative toxicity, hence the lower dosages used in the developmental toxicity studies listed. Albeit tested at lower dosages, only PNCB exhibited significant developmental toxicity in the comparative rat studies. Severe maternal toxicity, along with embryotoxicity, fetotoxicity and frank malformations were observed at the highest dosage tested. Only maternal toxicity and no embryotoxicity or fetotoxicity was observed at the mid dosage employed while the low dose selected was without treatment-related effect. As developmental effects were noted only at a dosage that produced significant maternal toxicity, PNCB is not considered to cause a primary effect on fetal development.

PNCB was toxic to rabbits in a developmental toxicity study (Nair et al, 1985). Frank maternal toxicity, including deaths, was observed at the highest dose tested, thus rendering determination of developmental toxicity impractical at this dosage level. There was no evidence of developmental toxicity observed at either of the two lower test levels used in this study.

ONCB, on the other hand, produced substantive maternal toxicity in rats at 100 mg/kg, but produced no evidence of either embryotoxicity, fetotoxicity or teratogenicity even at this level.

PNCB produced no evidence of adverse reproductive performance, including mating, fertility and pregnancy, littering or pup survival and development, in a Two-Generation rat Reproduction study using a top dosage which produced significant maternal toxicity (increased spleen weight, anemia, elevated blood methemoglobin levels) related to methemoglobinia following chronic dosing (Nair et al, 1989). PNCB, but not ONCB, affected reproductive outcomes in mice exposed during a series of continuous breeding cycles.

Based on the results of these studies and the NOEL's derived, an adequate margin of safety exists at the recommended occupational exposure limit established for the Chloronitrobenzenes.

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VII. ROBUST STUDY SUMMARIES
Appended

31

I U C L I D

Data Set

Existing Chemical	:	ID: 121-73-3
CAS No.	:	121-73-3
EINECS Name	:	1-chloro-3-nitrobenzene
EINECS No.	:	204-496-1
TSCA Name	:	Benzene, 1-chloro-3-nitro-
Molecular Formula	:	C6H4ClNO2

Producer Related Part

Company	:	Solutia Inc.
Creation date	:	04.04.2002

Substance Related Part

Company	:	Solutia Inc.
Creation date	:	04.04.2002
Memo	:	
Printing date	:	09.12.2002
Revision date	:	
Date of last Update	:	06.12.2002
Number of Pages	:	20
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

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	:	46 - ° C
Sublimation	:	
Method	:	other
Year	:	1995
GLP	:	no
Test substance	:	other TS
Method	:	experimental, method not reported.
Test substance	:	m-Chloronitrobenzene
Reliability	:	(2) valid with restrictions Also cited in reference document, Budavari, S. (ed). The Merck Index-an encyclopedia of chemicals, drugs and biologicals. Whitehouse Station, NJ. 1989. Cited as a Peer reviewed reference in HSDB (2002) for m-NCB.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (9)

2.2 BOILING POINT

Value	:	236 - ° C at 1013.25 hPa
Decomposition	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	other TS
Method	:	Not reported
Remark	:	Reported as 236 deg. C @ 760 mm Hg.
Reliability	:	(2) valid with restrictions Accepted reference standard and cited as a Peer reviewed reference in HSDB (2002) for m-NCB.
Flag	:	Critical study for SIDS endpoint

02.12.2002 (2)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	- .129 hPa at 25° C
Decomposition	:	
Method		other (measured)
Year	:	1994
GLP	:	no data
Test substance	:	other TS
Method	:	Not reported
Test substance	:	m-Chloronitrobenzene
Reliability	:	(2) valid with restrictions Original article cited as a Peer reviewed reference in HSDB (2002) for m-NCB.
Flag	:	Critical study for SIDS endpoint

05.12.2002 (3)

2.5 PARTITION COEFFICIENT

Log pow	:	= 2.49 - at 25° C
Method		OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year	:	1987
GLP	:	no data
Test substance	:	other TS
Method	:	Shake flask method; the experiments were conducted in triplicate. The test temperature was 25 +/- 0.5 deg. C. The concentration of the test substance in the aqueous phase was determined by UV/Visible Spectrophotometry, while the concentration in the n-octanol phase was calculated as the difference from the total amount added.
Test substance	:	m-Chloronitrobenzene with purity > 99% from Aldrich Chemical Co.
Reliability	:	(1) valid without restriction Conducted according to OECD guidance.
Flag	:	Critical study for SIDS endpoint

02.12.2002 (15)

2.6.1 WATER SOLUBILITY

Value	:	= 255.5 - mg/l at 25 ° C
Qualitative	:	moderately soluble (100-1000 mg/L)
Pka	:	at 25 ° C
PH	:	- at and ° C
Method	:	other
Year	:	1995
GLP	:	no
Test substance	:	other TS
Method	:	Group contribution methods allow for the estimation of water solubility based on the chemical structure of a given compound. Values assigned to substructural units (referred to as "fragments") are summed to give a final solubility for the entire compound. The fragment values used in this method were complied from the KWB1 (Klopman, Wang and Balthasar, 1992, J. Chem. Inf. Comput Sci. 32:474-482) and WYMW (Wakita, KM, Yoshimoto, S Miyamoto and H Watanabe. 1986, Chem. Pharm. Bull 34:4663-4681) group contribution methods. Additionally, as this method traditionally models liquids better than solids, a melting point term was included to improve the values generated for compounds considered solids (i.e. melting point >25 deg C).
Result	:	The estimated water solubility (Sw) of m-chloronitrobenzene was reported as log Sw[mol/l]= -2.79. Based on a molecular weight of 157.56 g/mol, the Sw=255.5 mg/l.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

06.12.2002 (9)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	other
Light source	:	Xenon lamp
Light spect.	:	- nm
Rel. intensity	:	- based on Intensity of Sunlight
Direct photolysis		
Halflife t1/2	:	-
Degradation	:	- 98 % after 5 hour(s)
Quantum yield	:	
Deg. Product	:	yes
Method	:	other (measured)
Year	:	1979
GLP	:	no data
Test substance	:	other TS
Method	:	One ml of m-chloronitrobenzene in n-hexane was put in 1 L reaction vessel, followed by substitution of n-hexane vapor with air or nitrogen free from nitrogen oxides. The TS deposited in the reaction vessel which corresponded to 1000 microliter gas if vaporized, was irradiated at 25 to 30 degrees C for five hours with the Xenon lamp (ozone-less type, Ushio, Co.). Disappearance of parent TS measured by HPLC; by-products measured by GC-MASS.
Result	:	Rate of disappearance was influenced by the intensity of light passing through either of two reaction vessels used in this experiment, i.e. pyrex and quartz. The rate of disappearance of MNCB in air free of nitrogen, when tested in pyrex and quartz vessels, respectively, was 3.6% and 89%. When MNCB was tested in nitrogen free of nitrogen oxides in pyrex and quartz vessels, respectively, disappearance rates were 8.9% and 98%. Reaction byproducts found in nitrogen-free air included: 3-chloro-2-nitrophenol, 3-chloro-6-nitrophenol and 3-chloro-4-nitrophenol. The reaction by-product in nitrogen fee from nitrogen oxides was m-chlorophenol.
Test substance	:	Laboratory synthesized, purity unstated.
Reliability	:	(2) valid with restrictions Established photodegradative properties expermentally in published literature article.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (8)

Type	:	other
Light source	:	
Light spect.	:	- nm
Rel. intensity	:	- based on Intensity of Sunlight
Deg. Product	:	
Method	:	other (calculated)
Year	:	2002
GLP	:	no
Test substance	:	
Method	:	Used AOP method in EPIWIN, 2002.
Result	:	Vapor phase m-chloronitrobenzene is susceptible to reaction with photochemically-produced hydroxyl (OH) radicals. The 2nd order rate constant for reaction with hydroxyl radicals was calculated as 0.1199E-12 cm3/molecule*sec). Based on 1.5E6 OH molecules/cm3 and assuming 12 hours of sunlight per day, the estimated photo-oxidation half-life is 89.2 days (~2140 hrs).
Reliability	:	(2) valid with restrictions Supplemental information as a measured value has been used to fulfill this HPV Endpoint. Use of estimation model recommended by US EPA.

06.12.2002 (5)

3.1.2 STABILITY IN WATER

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other
Air (level I)	:	7.96
Water (level I)	:	28.8
Soil (level I)	:	63
Biota (level II / III)	:	
Soil (level II / III)	:	.193
Method	:	other
Year	:	2002
Method	:	Level III Fugacity Model; EPIWIN, Version 3.10. Physical properties of m-chloronitrobenzene used as model input: water solubility=255.5 mg/L, vapor pressure=0.097 mm Hg, log Kow=2.46 and melting point=44.4 deg C. Emissions rates were 1000 kg/hr for each of the three main compartments, air, water and soil. Also estimated sediment compartment, listed in second soil entry.Persistence Time: 550 hr.
Reliability	:	(2) valid with restrictions Estimated values based on model recommended by US EPA.
Flag	:	Critical study for SIDS endpoint

02.12.2002 (5)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	anaerobic
Inoculum	:	anaerobic bacteria
Concentration	:	4µmol/l related to Test substance related to
Contact time	:	1 year
Degradation	:	50 - % after 3.2 day
Result	:	
Deg. Product	:	yes
Method	:	other
Year	:	1996
GLP	:	no data
Test substance	:	other TS
Method	:	This study was conducted to examine degradation of chloronitro compounds in sulfidogenic anaerobic sediment. A 2.0 x 10E-3 M stock solution of the test substance was prepared in methanol. The test medium was a slurry of estuarine sediment and river water from the mouth of the

Tsunumi river (Japan). Initial characteristics of the medium were: solids, 272 +/- 2.8 g/kg; nitrate, not detected (sediment), and 4 mg/l (water); sodium chloride, 1.7% (sediment) and 1.5% (water); sulfate, 1980 mg/l(sediment) and 1840 mg/l (water); pH of 5.6. Tests were conducted in screw-top test tubes. Twenty-five (25) tubes containing 5 ml test medium were prepared under nitrogen and stored for 1 week at room temperature to allow the test system to become anaerobic. Eight (8) tubes were autoclaved for use as sterile controls. Five (5) tubes were prepared with filtered river water only as the test medium, for use as sediment-free controls. Ten (10) ul of the stock solution was added to each tube yielding an initial concentration of 4 umole/l. All tubes were placed in an anaerobic chamber (10% H2, 10% CO2, 80% N2) at 25 deg. C. Test tubes were hand-mixed 3 times per week by inverting and were sampled for analysis at the beginning of incubation (t=0 hrs) and at various time intervals over the course of the test period (1 yr). Collected samples were frozen. The experiment was conducted in duplicate. Measurement of remaining test substance and identification of degradation products were conducted at the end of the test period using gas chromatography with mass-selective detection (GC-MS). Measured concentrations at each time interval were compared to measured concentrations at t=0 hrs. The first order rate constant (k) was determined by linear regression. The characteristic half-life (t1/2) was determined by the equation t1/2=(ln 2)k.

Result : Degradation of the parent compound was observed to occur without any lag time. The first order rate constant (k) was determined to be 0.216 +/- 0.096 per day. The half-life was determined to be 3.2 days. The coefficient of determination was reported as 0.89. Under the conditions of the test and in the presence of the sediment used, m-NCB was observed to degrade under anaerobic conditions in a two-stage process: (1) reduction of the nitro subsitituent group to form 3-chloroaniline and (2) removal of the chloro-substitutent group to form aniline.

Test substance : commercial grade mNCB from Tokyo Kasei Kogyo Col. Ltd, Japan; purity not specified but likely >99%.

Reliability : (2) valid with restrictions
Non standard test sytem, but well documented; individual data for test substance recoveries not provided.

Flag : Critical study for SIDS endpoint
02.12.2002 (13)

Type :
Inoculum : aerobic microorganisms
Concentration : 10mg/l related to Test substance
related to
Contact time : 64 day
Degradation : - % after
Result :
Deg. Product : not measured
Method : other
Year : 1961
GLP : no
Test substance : other TS
Method : The study was conducted to examine the effect of substituent groups on the potential degradation of benzene compounds by soil microorganisms. The test medium was a mixture of distilled water (1000 ml), K2HPO4 (1.6 g), KH2PO4 (0.4 g), NH4HO3 (0.5g), MgSO4.7H2O (0.2 g), CaCl2.2H2O (0.025 g), and FeCl3.6H2O (0.0023 g). The inoculum was a 1 % suspension of Niagara silt loam. Tests were conducted in 4 oz screw-cap bottles (45 m diameter x 80 mm high). The test substance (final substrate concentration = 10 mg/l), 40 ml of test medium, and 1 ml of inoculum were combined in a test vessel and incubated at 25 deg C in the dark. An unspiked control mixture (medium + inoculum) was prepared and incubated concurrently. Test vessels were prepared in duplicate. Two adiitional series of vessels were prepared to evaluate (a) the effect of

HgCl2 (8 mg) + Tween-80 (5.03-7) on degradation, and (b) the potential toxicity of the test substance to the inoculum. The toxicity control contained glucose (1%) + test substance. Test vessels were mixed and sampled for analysis at 3 and 6 hrs, and at 2, 4, 8, 16, 32, and 64 days after test initiation. Analyses were conducted using a UV spectrophotometer. Solutions containing the test substance were compared against the untreated control and degradation (given as cleavage of the benzene ring) would be demonstrated by a loss of UV absorbency.

Result : The wavelength reported for measuring the degradation of 3-chloronitrobenzene was 265 nm. Degradation of the test substance, i.e. breakdown of the benzene ring, was observed to be difficult and did not completely occur during the test period. Under the conditions of the test, degradation of the monochloronitro group of compounds by soil microorganisms, was reported to be > 64 days. Additionally, HgCl2 did not appear to affect the degradation rate of the test substance, and the test substance did not appear to be toxic (i.e. after the rate of microbial development) to the inoculum.

Test substance : m-NCB, purity unspecified.

Reliability : (2) valid with restrictions
Provided as Supplementary information for this HPV Endpoint; study was non-standard and predated GLPs, but was well documented.

02.12.2002 (1)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
LC50	:	= 18.8 -
Method	:	other
Year	:	1986
GLP	:	no data
Test substance	:	other TS
Method	:	US EPA methodology (unspecified which one); Fathead minnows used in the tests were cultured from brood stock provided by the USEPA Environ. Res. Lab (Duluth) and the University of Wisconsin-Superior. Fish used were 33 days old and had a calculated mean weight of 0.148 g. Fish were not fed during the test. The toxicity test was conducted using 19-L vessels. Test volume was 7.3 L. The control/dilution was unfiltered Lake Superior water. Water quality parameters measured during the test included: total hardness, 45.3 +/- 0.58 mg/l (as CaCO3); alkalinity, 42.8 +/- 0.45 mg/l (as CaCO3), dissolved oxygen, 7.0 +/- 0.21 mg/l, temperature, 23 +/- 0.11 deg. C., and pH, 7.45 +/- 0.06. Test concentrations were analyzed daily (single sample; alternating replicates) by GLC. Two replicates of 25 fish each were exposed to the control/dilution water and to each of five measured concentrations of the test substance. Mortality and abnormal signs of behavior were recorded at 3, 6, 24, 48, 72 and 96 hrs.LC50 tabulated using the Trimmed Spearman-Karber method.
Result	:	Average measured concentrations (test replicate): control= <0.25 mg/L (limit of detection), Dosage # 1 - 3.15 mg/l (replicate 1) and 2.8 mg/l (replicate 2), Dosage # 2 - 5.05/4.5 mg/l; Dosage # 3 - 7.25/10.0 mg/l; Dosage # 4 - 14.4/12.9 mg/l; Dosage # 5 - 24.1/22.4 mg/l. The concentration of test material was maintained during the exposure period (percent recovery was reported as 95.6 +/- 1.9 %). Results of the 96 hr acute toxicity test by concentration: No deaths at 0 or Dosages 1-4; at Dosage # 5 partial mortality was observed betwen 3-6 hr after treatment. 100 % mortality was seen in replicate 1 @ 24 hr while 92 % mortality was seen in replicate 2 at 24 hrs. No further deaths were observed later in the study period. The 96-h LC50 was reported as 18.8 mg/L Affected fish were reportedly hypoactive, lost schooling behavior and lost equilibrium prior to death.
Test substance	:	m-CNB from Aldrich Chem. Co. with purity of 98%.
Reliability	:	(2) valid with restrictions Well documented study conducted under EPA test guidelines; no EC50 determined, nor were CI measured (unreliable by stat. method used)
Flag	:	Critical study for SIDS endpoint

02.12.2002 (7)

Type	:	
Species	:	other
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
LC50	:	= 43.2 -
Method	:	other
Year	:	2002
GLP	:	no
Test substance	:	no data
Method	:	calculated using ECOSAR.
Result	:	An acute fish 96-h LC50 was calculated using ECOSAR from US EPA; the

SAR for esters was used. The structure was determined from the CAS RN, as stored in the accompanying database of SMILES notations within ECOSAR.

Reliability : (2) valid with restrictions
Provided as Supplementary information; model used has been accepted by US EPA.

06.12.2002 (14)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	
EC50	:	c = 47.7 -
Method	:	other
Year	:	2002
GLP	:	no
Test substance	:	no data
Method	:	An acute Daphnia 48-h LC50 was calculated using ECOSAR. The SAR for esters was used. The structure was determined from the CAS RN, as stored in the accompanying database of SMILES notations within ECOSAR.
Reliability	:	(2) valid with restrictions Estimate obtained from US EPA recommended model and supported by additional literature data included in this section. Estimate obtained from US EPA recommended model and supported by additional literature data included in this section.
Flag	:	Critical study for SIDS endpoint

02.12.2002 (14)

Type	:	
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no data
EC50	:	= 20 -
Method	:	other
Year	:	1980
GLP	:	no data
Test substance	:	other TS
Method	:	NEN 6501 -"Determination of acute toxicity with Daphnia magna". Dutch Standardization Organization, Rijswijk, The Netherlands. The 48-h acute test was conducted with three CNB isomers. The LC50 and 95% confidence limits were determined by the Litchfield and Wilcoxon (1949) method.
Result	:	LC50=20 mg/l with CI of 10-32 mg/L. Relative toxicity of the three CNB isomers were (high to low): para>meta>ortho.
Test substance	:	m-NCB purity of 99%, obtained from Merck.
Reliability	:	(4) not assignable Provided as Supplemental data, in that study, as published, provides insufficient individual data to allow higher categorization. Provided as Supplemental data, in that study, as published, provides insufficient individual data to allow higher categorization.

06.12.2002 (10)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	other algae
Endpoint	:	
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	
EC50	:	c = 30.6 -
Method	:	other
Year	:	2002
GLP	:	no
Test substance	:	
Method	:	Method of calculation of an acute green algal 96-h LC50 used ECOSAR from USEPA. The SAR for esters was used. The structure was determined from the CAS RN, as stored in the accompanying database of SMILES notations within ECOSAR.
Reliability	:	(2) valid with restrictions Estimate obtained from US EPA recommended model and supported by additional literature data included in this section.
Flag	:	Critical study for SIDS endpoint

02.12.2002 (14)

Species	:	Chlorella pyrenoidosa (Algae)
Endpoint	:	other
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no data
EC50	:	= 1.9 -
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	1984
GLP	:	no data
Test substance	:	other TS
Method	:	The 96-h EC50 for effects on yield and 95% confidence limits were determined by the method of Kooyman et al, 1983 and followed guidance of OECD # 201. Yield was the point of measurement.
Result	:	The 96-h EC50=1.9 mg/l with confidence limits of 1.5-2.6 mg/l.
Test substance	:	m-NCB with purity of 99% obtained from Merck.
Reliability	:	(4) not assignable Provided as Supplemental data, in that study, as published, provides insufficient individual data to allow higher categorization.

06.12.2002 (10)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

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

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	50
Vehicle	:	
Value	:	= 400 - mg/kg bw
Method	:	OECD Guide-line 401 "Acute Oral Toxicity"
Year	:	1983
GLP	:	yes
Test substance	:	other TS
Method	:	Used 5 rats per sex per each of 6 dose groups: 0, 200, 251, 316, 398,and 501 mg/kg; test article was administered undiluted once via gavage and animals observed for up to 14 days. Daily observations were made for mortality and clinical signs of toxicity. Body weights were recorded prior to start of the study and weekly thereafter. Necropsies were performed on all animals. Food and water were given ad libitum and humidity and temperature controlled. LD50 and 95% CI calculated according to method of deBeer, 1945, J. Pharmacol. Experimen. Ther. 85:1.
Result	:	Oral LD50 calculated as 400 (95% Confidence Limits of 350-470) mg/kg. Deaths occurring at each dose level included: At 200 mg/kg - 0/5M, 0/5F; at 251 mg/kg-2/5M, 0/5F, at 316 mg/kg-1/5M, 2/5F, at 398 mg/kg-5/5M, 0/5F, at 501 mg/kg-5/5M, 5/5F. Deaths occurred almost universally within the first 24 hr of dosing. Generalized signs of toxicity included lethargy, weakness and collapse before death. No other overt signs of toxicity were seen. At necropsy of decedents the following observations were made: hemorrhagic lungs, discoloration of the liver, kidney and spleen (2 cases) and gastrointestinal irritation. No discernable observations were noted for survivors necropsied after 14 days on test.
Test substance	:	Purity of 98%.
Reliability	:	(1) valid without restriction Meets OECD guidance and conducted under GLPs
Flag	:	Critical study for SIDS endpoint

02.12.2002 (12)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	no data
Strain	:	other
Route of admin.	:	oral unspecified
Exposure period	:	up to 7 months
Frequency of treatment	:	daily
Post obs. period	:	
Doses	:	60 mg/kg for 20 days; 5, 0.025, 0.005 and 0.0025 mg/kg/d for 7 months
Control group	:	yes
NOAEL	:	= .005 - mg/kg bw
Method	:	other
Year	:	1967
GLP	:	no data
Test substance	:	no data
Method	:	Peroral treatment of 20 albino rats at 60 mg/kg for 20 days, followed by peroral administration of MNCB to groups of rats at 5, 0.025, 0.005 and 0.0025 mg/kg/d for 7 months. Measured indices reportedly included hematology, liver function (blood and urine) and peripheral blood pathology.
Result	:	4/20 rats treated with 60 mg/kg MNCB for 20 days died. Groups of rats treated for 7 months exhibited marked changes in peripheral blood. Methemoglobin levels were increased within the first month of testing in the HD group; elevations occurred in groups treated with 0.025 mg/kg MNCB or higher. Hemoglobin was reduced and reticulocytes, serum alkaline phosphate and urinary bilirubin were elevated along with presence of Heinz bodies in erythrocytes at dosages of 0.025 mg/kg/d and above. The NOEL was 0.005 mg/kg/d.
Conclusion	:	Comparative study using ONCB, MNCB and PNCB. Concluded that PNCB was the most toxic isomer following systemic exposure, MNCB was intermediate, and ONCB was the least systemically toxic of the three isomers tested. All isomers exhibited essentially the same pattern of toxicity.
Reliability	:	(4) not assignable Supplemental information, as this report provides but a summary of results without sufficient detail to be classified higher.

06.12.2002 (4)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	S. typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538
Concentration	:	25.6, 51.2, 102.4, 204.8, 409.6, 819.2, 1638.4 and 3276.8 ug/plate
Cycotoxic conc.	:	3276.8 ug/plate
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1977
GLP	:	no
Test substance	:	other TS
Method	:	Design consistent with but preceeded OECD Testing and GLP guidance. Tester strains obtained from B. Ames, S9 fraction came from PCB injected male SD rats, test article dissolved in DMSO for use in pour-plate method for quantitative determination of mutagenic activity according to Ames. A negative control group used 0.05 ml DMSO while positive controls tested were: MNNG, 2-NF, 9-AA and 2-AA (S9 added only). All tests were performed in duplicate and repeated at least three times. A positive

response was identified when colonies of his+ revertants on test article-treated plates were more than twice the number of revertant colonies on the control plates.

Result : No 2X mutagenic response was observed at any treatment level for any of the tester strains, with or without S9. Positive control agents performed as expected. Significant cytotoxicity was observed at 3276.8 ug/plate, based on depletion of background lawn.

Test substance : Obtained from Tokyo Kassei Kogyo Co. Ltd.

Reliability : (2) valid with restrictions
Study conducted consistent with but prior to development of US GLPs effective 6/79; project was conducted in association with US EPA. Study results are confirmed in additional literature references (Simmon et al. 1977. Dev. Toxicol. Environ. Sci. 2:249-258 and Suzuki et al. 1983. Mut. Res. 120:105-110).

Flag : Critical study for SIDS endpoint

06.12.2002 (11)

Type : Cytogenetic assay

System of testing : CHO (Chinese Hamster Ovary) cells in vitro assay

Concentration : 0, 50, 160, and 500 ug/ml

Cycotoxic conc. :

Metabolic activation : with and without

Result : negative

Method : other

Year : 1987

GLP : yes

Test substance :

Method : Test consisted of concurrent and positive (TEM) controls and at least 3 doses of test material. A single flask per dose was used. Cells were incubated in McCoy's 5A medium with test agent for 12 hrs (cells were treated with test agent and S9 for 2 hrs), colcemid added and cells incubated for an additional 2 hrs and harvested. 100 first-division metaphase cells were scored blind from prepared slides for each dose level. Classes of aberrations were recorded and included simple, complex and other abnormalities. Statistical analyses (linear regression analysis and, for absolute increases, methodology of Margolin et al 1983) was conducted on both the dose-response curve and individual dose points, significance was determined as $p<0.05$ for single data points and $p<0.015$ for trend.

Result : Did not induce Chrom Abs in CHO cells with or without metabolic activation at any test level.

Reliability : (2) valid with restrictions
Combined Chromosomal Aberration and SCE study followed NTP study design. Was well documented and useful for regulatory purposes. SCE study portion was reported as equivocal as "the very slight increase in SCEs without S9 is unlikely to be meaningful despite the positive trend test; the other results were negative".

Flag : Critical study for SIDS endpoint

02.12.2002 (6)

5.6 GENETIC TOXICITY 'IN VITRO'

5.7 CARCINOGENITY

5.8 TOXICITY TO REP RODUCTION

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.10 OTHER RELEVANT INFORMATION

5.11 EXPERIENCE WITH HUMAN EXPOSURE

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7.1 END POINT SUMMARY

7.2 HAZARD SUMMARY

7.3 RISK ASSESSMENT

I U C L I D

Data Set

Existing Chemical	:	ID: 88-73-3
CAS No.	:	88-73-3
EINECS Name	:	1-chloro-2-nitrobenzene
EINECS No.	:	201-854-9
TSCA Name	:	Benzene, 1-chloro-2-nitro-
Molecular Formula	:	C6H4ClNO2

Producer Related Part

Company	:	Solutia Inc.
Creation date	:	04.04.2002

Substance Related Part

Company	:	Solutia Inc.
Creation date	:	04.04.2002
Memo	:	
Printing date	:	09.12.2002
Revision date	:	
Date of last Update	:	06.12.2002
Number of Pages	:	26
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

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	:	= 32.5 ° C
Sublimation	:	
Method	:	other
Year	:	1996
GLP	:	no data
Test substance	:	other TS
Method	:	not reported
Test substance	:	o-Chloronitrobenzene
Reliability	:	(2) valid with restrictions Obtained from accepted reference text and value cited as Peer reviewed in HSDB (2002) for o-chloronitrobenzene and in EPA draft CHIP for oNCB (1983)
Flag	:	Critical study for SIDS endpoint

02.12.2002 (19)

2.2 BOILING POINT

Value	:	= 245.7 ° C at
Decomposition	:	
Method	:	other
Year	:	1996
GLP	:	no data
Test substance	:	other TS
Method	:	not reported
Test substance	:	o-chloronitrobenzene
Reliability	:	(2) valid with restrictions Obtained from accepted reference text and value cited as Peer reviewed in HSDB (2002) for o-chloronitrobenzene and in EPA draft CHIP for oNCB (1983)
Flag	:	Critical study for SIDS endpoint

02.12.2002 (19)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= .0575 hPa at 20° C
Decomposition	:	
Method		other (measured)
Year	:	1999
GLP	:	no data
Test substance	:	other TS
Method	:	not reported
Test substance	:	o-Chloronitrobenzene
Reliability	:	(2) valid with restrictions Obtained from accepted reference text and similar value cited in EPA draft CHIP for oNCB (1983)
Flag	:	Critical study for SIDS endpoint

12.11.2002 (1)

2.5 PARTITION COEFFICIENT

Log pow	:	= 2.24 at ° C
Method		other (measured)
Year	:	1971
GLP	:	no data
Test substance	:	other TS
Method	:	not reported
Test substance	:	o-Chloronitrobenzene
Reliability	:	(2) valid with restrictions Obtained from standard reference text and cited as Peer reviewed in HSDB (2002) for o-NCB and the EPA draft CHIP (1983) for o-NCB.
Flag	:	Critical study for SIDS endpoint

02.12.2002 (8)

2.6.1 WATER SOLUBILITY

Value	:	= 307.2 mg/l at 25 ° C
Qualitative	:	moderately soluble (100-1000 mg/L)
Pka	:	at 25 ° C
PH	:	at and ° C
Method	:	other
Year	:	1995
GLP	:	no data
Test substance	:	other TS
Method	:	calculated; group contribution method. Group contribution methods allow for the estimation of water solubility based on the chemical structure of a given compound. Values assigned to substructural units (referred to as "fragments") are summed to give a final solubility for the entire compound. The fragment values used in this method were compiled from the KWB1 (Klopman, G,S Wang and DM Balthasar. 1992. J. Chem. Inf. Comput Sci. 32:474-482) and WYMW (Wakita, K, M Yoshimoto, S Miyamoto and H Watanabe. 1986. Chem. Pharm Bull 34:4663-4681) group contribution methods. Additionally, as this method traditionally models liquids better than solids, a melting point term was included to improve the values generated for compounds considered solids.
Result	:	The estimated water solubility (Sw) of o-Chloronitrobenzene was reported as log Sw [mol/l]= -2.71. Based on a molecular weight of 157.56 g/mol, Sw = 307.2 mg/l.
Test substance	:	o-Chloronitrobenzene.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

06.12.2002 (7)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	other
Light source	:	Xenon lamp
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Direct photolysis		
Halflife t1/2	:	
Degradation	:	66 % after 5 hour(s)
Quantum yield	:	
Deg. Product	:	yes
Method	:	other (measured)
Year	:	1979
GLP	:	no data
Test substance	:	other TS
Method	:	One milliliter of o-chloronitrobenzene in n-hexane was put in 1 liter reaction vessel (either pyrex or quartz), followed by substitution of n-hexane vapor with air or nitrogen free from nitrogen oxides. TS was deposited in the reaction vessel, which corresponds to 1000 microliter gas if vaporized, and was irradiated at 25 to 30 degrees C for 5 hours with the Xenon lamp (ozone-less type, Ushio Co.). Disappearance rate measured via HPLC of parent TS. By-product identification was by GC-MASS.
Result	:	Rate of disappearance was influenced by the intensity of light passing through either of two reaction vessels used in this experiment, i.e. pyrex and quartz. The rate of disappearance of ONCB in air free of nitrogen oxides, when tested in pyrex and quartz vessels, respectively, was 4.3% and 66%. When ONCB was tested in nitrogen free of nitrogen oxides in pyrex and quartz vessels, respectively, disappearance rates were 9.5% and 93%. The reaction by-products in air free from nitrogen oxides were 2-chloro-6-nitrophenol and 2-chloro-4-nitrophenol. The reaction by-product in nitrogen free from nitrogen oxides was o-chlorophenol.
Test substance	:	Laboratory synthesized, purity unspecified.
Reliability	:	(2) valid with restrictions Published literature source which corraborates photodegradation potential of ONCB.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (5)

Type	:	other
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Direct photolysis		
Halflife t1/2	:	= 62.5 day
Degradation	:	% after
Quantum yield	:	
Deg. Product	:	
Method	:	other (calculated)
Year	:	2002
GLP	:	no
Test substance	:	
Method	:	AOPWIN, v. 1.90; vapor phase of o-chloronitrobenzene is susceptible to reaction with photochemically-produced hydroxyl (OH) radicals.
Result	:	The 2nd order rate constant for reaction with hydroxyl radicals was calculated as 0.1714E-12 cm3/(molecule*sec.). Based on 1.5E6 OH molecules/cm3 and assuming 12 hours of sunlight per day, the estimated photo-oxidation half-life is 62.4 days (~1500 hrs).
Reliability	:	(2) valid with restrictions Supplemental information as the previous study fultills this HPV data

requirement. Estimated value based on model recommended by US EPA.

06.12.2002 (4)

3.1.2 STABILITY IN WATER

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other
Air (level I)	:	6.51
Water (level I)	:	33.5
Soil (level I)	:	59.8
Biota (level II / III)	:	
Soil (level II / III)	:	.162
Method	:	other
Year	:	2002
Method	:	Level III Fugacity Model employed using EPIWIN, v. 3.10. Physical properies for ortho-chloronitrobenzene have been cited in this report and include: water solubility = 307.2 mg/l; vapor pressure = 0.043 mm Hg (~ 0.0575 hPa); log Kow = 2.24 and melting point of 32 deg. C. Emissions rates were set at 1000 kg/hr for air (half life of 1500 hr), water (half life of 900 hr), soil (half life of 900 hr) and sediment (half life of 3600 hr). Persistence Time: 576 hrs. The second soil listing is the projected sediment loading.
Reliability	:	(2) valid with restrictions Estimated values based on model recommended by US EPA with ONCB specific entry data.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (4)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge, domestic, non-adapted
Concentration	:	1mg/l related to Test substance 10mg/l related to Test substance
Contact time	:	10 month
Degradation	:	= 11 - 48 % after 24 hour(s)
Result	:	
Deg. Product	:	
Method	:	other
Year	:	1965
GLP	:	no

Test substance	:	other TS
Method	:	SCAS test conducted over a 10-month period per J. Am. Oil Chemists Society methods (JAOCS, 1965, 42:986 and JAOCS, 1965, 46:432). Feeding rate started at 1 mg/24-h and raised in 1 mg increments to 5 mg over 28 days and held at 5 mg/24-h for 4 months, then raised to 10 mg/24-h. Twenty mL samples of mixed liquor (activated sludgte + liquor) were taken 1 hour after each addition and at the end of the aeration cycle via sidearm stopcock. The mixed liquor was extracted and analyzed via UV spectroscopy according to laboratory SOPs. Spike recovery experiments were 99.1 +/- 4.7%. Inoculum came from municiple sludge.
Result	:	Average disappearance rate, days 75-120 (5 mg feed level, high aeration rate) was 10.6 +/- 9.4 % over a 24-h cycle; over the next 60 days (5 mg feed rate, high aeration rate) it was 37.5 +/- 8.8 % over a 24-h cycle, and over the last two weeks (10 mg feed level, low aeration) it averaged 47.7 +/-8.1% per 24-hr cycle.
Test substance	:	Commercial grade ONCB with purity of 98.9%.
Reliability	:	(2) valid with restrictions Well documented study which conformed to pre-OECD/EPA guidance for SCAS testing; methodology used subsequently codified into test guidance for # 302A.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (16)

Type	:	aerobic
Inoculum	:	other
Concentration	:	.0961mg/l related to Test substance related to
Contact time	:	56 day
Degradation	:	6 % after 56 day
Result	:	
Deg. Product	:	
Method	:	other
Year	:	1971
GLP	:	no
Test substance	:	other TS
Method	:	River Die-Away test (RDA). River water was obtained from the Mississippi River near St. Louis, Mo. USA. Settled water (2 days) was added (250 ml) to 500 ml narrow-mouth bottles. Distilled water controls (with test substance) were prepared similarly to assess sorption to glass and volatilization. TS was added in 5 microliter volumes prepared with 5% (w/v) ethanol. Bottles were sealed with foil-lined caps and stored at room temperature in the dark. A positive control (LAS Reference # 2-dodecene-1) was prepared similarly and used to verify the biological activity. Periodically, chemical analyses were made by sacrificing a bottle with TS and a control. A 25 mL aliquot of hexane was injected into the bottle, the bottle vigorously shaken, and the phases allowed to separate. A portion of the hexane was collected, transferred to a 2 mL cell and the UV absorption determined. Recoveries of spiked samples for the TS were 91.6%.
Result	:	Losses from the distilled water control were insignificant (0.996 mg/L at day 0 and 1.004 mg/L at day 56. TS concentration was 0.961 mg/L at day 0 and 0.904 mg/L at day 56 (loss of 5.93% due to biodegradation in 56 days).
Test substance	:	Commercial grade ONCB with purity of 99%.
Reliability	:	(2) valid with restrictions Supplemental information as previously reported study fulfills HPV endpoint.

06.12.2002 (16)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	semistatic
Species	:	Poecilia reticulata (Fish, fresh water)
Exposure period	:	14 day
Unit	:	mg/l
Analytical monitoring	:	yes
LC50	:	= 30.03
Method	:	
Year	:	1981
GLP	:	no data
Test substance	:	other TS
Method	:	Methodology fully summarized in Koenemann, 1981. Toxicology 19:209-221. Guppies used in testing were raised in the laboratory of the Department of Veterinary Pharmacology, Pharmacy, and Toxicology, University of Utrecht, The Netherlands. Fish used in the 14-day toxicity tests were 2-3 months old at test initiation. Tests were conducted in 1.5 liter standard jars. Fish were acclimated for a minimum of 12 days prior to testing. The test water used was prepared standard water, corresponding to very soft tap water. Test water had a hardness of 25 mg/l (as CaCO3). Water quality parameters measured during the test (at least 4 days over the exposure period, before and after solution renewal) included oxygen content of >4.5 mg/L; temperature of 21-23 deg.C. and pH of 6.8-7.2. Test concentrations were analyzed (at least every 4 days over the exposure period, before and after solution renewal), by gas chromatography with both electron capture and flame-ionization detection (GC-ECD/FID). Ten fish each were exposed to a geometric progression of test substance concentrations. Mortality and abnormal signs of behavior were recorded over the exposure period. LC50 value was calculated from mortality data by logit transformation and were based on nominal concentrations.
Result	:	The 14-d LC50 for ONCB was 2.28 umoles/l (based on a molecular weight of 157.6 g/mol, the LC50 can be expressed as 30.03 mg/l). Authors reported that measured concentrations of test solutions were at least 80% of nominal. Test organisms generally showed loss of balance, lethargy and increased appetite at low concentrations of the test substance. At high concentrations, cyanosis was noted in some test organisms.
Test substance	:	Commercial grade o-chloronitrobenzene with purity of 99%.
Reliability	:	(2) valid with restrictions Well documented study which lacks some individual information (i.e. lengths/weights of fish); actual concentrations not listed and no information on control group reported.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (3)

Type	:	other
Species	:	other
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
LC50	:	c = 69.5
Method	:	other
Year	:	2002
GLP	:	no
Test substance	:	other TS
Method	:	calculated using ECOSAR
Result	:	An acute fish 96-hr LC50 was calculated using ECOSAR from US EPA; the SAR for esters was used. The structure was determined from the CAS RN, as stored in the accompanying database of SMILES notations within ECOSAR

Test substance	:	o-Chloronitrobenzene
Reliability	:	(2) valid with restrictions Provided as Supplementary information; model used has been accepted by US EPA.

12.11.2002 (18)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
NOEC	:	= 12.5
EC50	:	= 41
Method	:	other
Year	:	1975
GLP	:	no
Test substance	:	other TS
Method	:	Followed EPA method # 660/3-75-009. Ten 24-h old D. magna were tested at 24 deg. C in a series of three replicates per test concentration. Test concentrations were 6.25, 12.5, 25, 50, 100 mg/L plus clean water and solvent (0.5 mg/L DMF) controls. Tests were conducted in well water from St. Peters, Mo. USA. Test concentrations were not measured. Daphnids were not fed. Tests were conducted in 250-mL beakers containing 200 mL of solution. Dissolved oxygen was monitored to ensure the concentration did not fall below 2 mg/L before the end of the test. Water quality was measured according to SOPs for dissolved oxygen, pH, alkalinity, hardness and temperature and no significant changes were observed in any parameter.The estimated EC50 and 95% confidence limits were determined using EPA statistical procedures.
Result	:	There were no control mortalities, plus no partial and total mortalities in the test vessels with test substance. The 24-h EC50 (95%CL) = 45 (25-100) mg/L; the 48-h EC50 (95%CL) = 41 (35.7-47-6) mg/L.; The NOEC = 12.5 mg/L. Water temp. was 24 deg. C in all test vessels, pH ranged between 7.0 - 7.2, alkalinity measured between 146-296 mg/L, dissolved oxygen ranged between 7.6-10.7 and hardness was between 162-304 mg/L.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

06.12.2002 (15)

Type	:	
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
EC50	:	c = 95.7
Method	:	other
Year	:	2002
GLP	:	no
Test substance	:	other TS
Method	:	An acute Daphnia 48-h LC50 was calculated using ECOSAR, from the US EPA. The SAR for esters was used. The structure was determined from the CAS RN, as stored in the accompanying database of SMILES notations within ECOSAR.
Reliability	:	(2) valid with restrictions Provided as supplemental information in that an acceptable study meets this HPV endpoint. Provided as supplemental information in that an acceptable study meets this HPV endpoint.

12.11.2002 (18)

Type	:	
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	
EC50	:	= 24
Method	:	other
Year	:	1980
GLP	:	no data
Test substance	:	other TS
Method	:	NEN 6501- "Determination of acute toxicity with Daphnia magna". Dutch Standardization Organization, Rijswijk, The Netherlands. The 48-h acute test was conducted with three CNB isomers, including o-nitrochlorobenzene. The LC50 and 95% confidence limits were determined by the Litchfield and Wilcoxon (1949) method.
Result	:	LC50=24 mg/L with CL of 18-32 mg/L. Relative toxicity of the three CNB isomers were (high to low): para>meta>ortho.
Test substance	:	o-nitrochlorobenzene with purity of 99%, obtained from Merck.
Reliability	:	(4) not assignable Provided as supplemental data in that this study, as published, provides insufficient individual data to allow higher categorization. Provided as supplemental data in that this study, as published, provides insufficient individual data to allow higher categorization.

06.12.2002 (9)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	biomass
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no data
EC10	:	m = 11
EC50	:	m = 34
Method	:	other
Year	:	1988
GLP	:	no data
Test substance	:	other TS
Method	:	DIN 38412, Part 9 - The green alga S. subspicatus (Strain 8681 SAG) was used to conduct a modified cell multiplication inhibition test. A stock solution of the test substance was prepared in double-distilled water and diluted to prepare a series of test concentrations ranging from 0.80-100 mg/L. The test was conducted in capped 250 ml Erlenmeyer flasks. Eight (8) replicates of each concentration were tested. Flasks were inoculated with the cell suspension (cell concentration of 10E5 cells/ml in each flask), placed on a white surface, protected from sunlight, shaken daily, and exposed to constant artificial lighting. The temperature was maintained at 24 +/- 1 deg C. and the relative humidity was 50%. A control group (8 replicates) was tested concurrently. On each measurement day, 50 ml were collected from each of two flasks from each test concentration or the control. The extinction value of the monochromatic radiation (578 nm wavelength) of the cell suspension was determined for each test concentration and the control. Samples were collected and measurements were made at the beginning of the test and after 24 and 48 hrs. Biomass determination was based on measurement of optical density (turbidity). EC values were determined by regression analysis.
Result	:	Mean measured values of control group at 48 hrs were : extinction value-0.068; Biomass-3.6x10E5 cells/ml. Results of the cell multiplication inhibition test of TS were: 48-hr Biomass EC10=11 mg/L; 48-h Biomass

EC50= 34 mg/L The 48-h average specific growth rate EC10= 19 mg/L.; the 48-h average specific growth rate EC50= 75 mg/L.

Test substance	:	o-chloronitrobenzene.
Reliability	:	(2) valid with restrictions Small deviations from standard study design, including less duration used, and limited information presented on each test concentration at each measurement point.
Flag	:	Critical study for SIDS endpoint

02.12.2002 (6)

Species	:	other algae
Endpoint	:	
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	
EC50	:	c = 48
Method	:	other
Year	:	2002
GLP	:	no
Test substance	:	other TS
Method	:	Method of calculation of an acute green algal 96-h LC50 used ECOSAR from USEPA. The SAR for esters was used. The structure was determined from the CAS RN, as stored in the accompanying database of SMILES notations within ECOSAR.
Reliability	:	(2) valid with restrictions Provided as Supplemental information as a previous study fulfills this HPV endpoint.

02.12.2002 (18)

Species	:	Chlorella pyrenoidosa (Algae)
Endpoint	:	other
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no data
EC50	:	m = 6.9
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	1984
GLP	:	no data
Test substance	:	other TS
Method	:	The 96-h EC50 for effects on yield and 95% confidence limits were determined by the method of Kooyman et al, 1983 and followed guidance of OECD # 201.
Result	:	The 96-h EC50 and confidence limits were 6.9 (5.7-8.4) mg/L.
Test substance	:	o-Chloronitrobenzene obtained from Merck with purity of 99%.
Reliability	:	(4) not assignable Provided as Supplemental data, in that this study, as published, provides insufficient individual data to allow higher categorization.

06.12.2002 (9)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

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

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	20
Vehicle	:	other
Value	:	560 mg/kg bw
Method	:	other
Year	:	1973
GLP	:	no
Test substance	:	other TS
Method	:	Administered by single oral gavage in corn oil as vehicle to groups of male and female (total of 5 mixed sex per group) rats given 398, 501, 631 and 764 mg/kg ONCB. Clinical signs of toxicity and deaths were recorded daily for the 7-day observation period. Body weights were recorded on test days 0 and 7. Necropsies were performed after death and on all rats surviving to day 7. Food and water were provided ad libitum and temp., humidity and light were controlled. LD50 value and CI were calculated by method of deBeer, J. Pharmacol Experiment. Ther. 86:1.
Result	:	OLD50 = 560 mg/kg with CI of 535-585 mg/kg. Deaths observed were: 1/5 @ 398 mg/kg, 2/5 @ 501 mg/kg, 4/5 @ 631 mg/kg, and 5/5 @ 764 mg/kg. Deaths occurred during days 1-4 of testing with the majority occurring within the first two days of the test. Toxicologic signs observed were increased weakness and ocular discharge. Decedents exhibited hemohaggic lungs and discolored livers, spleens, and kidneys at necropsy. After 7 days, survivors exhibited lung congestion and darkened spleens and kidneys at necropsy.
Test substance	:	Used commercial grade, > 99% pure, dosed in 10% corn oil and heated to 115 deg F before gavaging.
Reliability	:	(2) valid with restrictions Conducted with fewer animals than OECD guideline 401 and prior to inception of US EPA GLPS. The shorter duration for observation than found in the OECD test guidance for this study type did not affect its ourcome or the conclusions drawn, as all deaths occurred within the first two days of the study with surviving animals recovering within the latter portion of this study. Test results (OLD50 values ranging between 144-560 mg/kg) are consistent with additional studies for this endpoint in the ECB IUCLID (2002) for ONCB
Flag	:	Critical study for SIDS endpoint

02.12.2002 (17)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	inhalation
Exposure period	:	6 hr/day
Frequency of treatment	:	5 days per week for 13 weeks
Post obs. period	:	none
Doses	:	0, 1.1, 2.3, 4.5, 9 or 18 ppm
Control group	:	yes
NOAEL	:	< 1.1 ppm
Method	:	OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year	:	1989
GLP	:	yes
Test substance	:	other TS
Method	:	Groups of 10 male and 10 female F-344 rats were exposed in whole body stainless steel and glass chambers to vapors containing 0, 1.1, 2.3, 4.5, 9 or 18 ppm ONCB for 6 hr/day, 5 days per week, for 13 weeks. Vapor was generated by heating ONCB first in a water bath, then in a hot-oil bath and passing metered nitrogen over the test material prior to introduction into the test chamber. Levels of flow were registered using automated data acquisition and control systems. Air concentrations were measured using GC/EC. Due to the low volatility of ONCB, it was concluded that the level achieved in a vapor state was essentially the maximum technically achievable. Animals were individually caged, food and water administered ad libitum, and a 12 hr light;dark cycle employed. All animals were assessed for morbidity and mortality daily, and weekly examined for clinical toxicity and recording of body weights. At termination of the study (13 weeks) all animals were necropsied and a full set of over 40 tissues and organs were examined microscopically for all high dose and control animals; target organs were examined for animals from lower dose groups. Organ weights and relative weights were assessed for all animals after 13 weeks of testing and included the following organs: heart, kidney, lung, liver, spleen, testis and thymus. The following hematology parameters were assessed on study day 1 (Methemoglobin only), 4, 23 and at 13 weeks from all rats from each study group: HCT, HGB, RBC, RETIC, MCV, MCH, MCHC, PLAT, WBC, MET, and WBC differentials. Similarly, the following clinical chemistry parameters were measured from all rats at similar time points as hematology: BUN, CREAT, TPROT, ALB, GLOB, ALT, AP, CK, SDH and bile acids.Williams parametric multiple comparison procedure was employed to statistically assess group-wise comparison of organ and body weights. Shirley's test for nonparametric analysis was used for clinical chemistry and hematology assessments. P<0.05 and 0.01 were used in all cases.
Remark	:	In a satellite group used to assess reproductive parameters, no significant changes were seen in groups of females, but lower epididymal wts, spermatid heads /testis and spermatid count in the 18 ppm male dose group was noted.
Result	:	Following are treatment-related effects noted at each dose group: 1.1 ppm - Males - increased MET, SDH, and RE (Respiratory Epithelium) hyperplasia; females - increased. MET and RE hyperplasia. 2.3 ppm -

Males - increased MET,SDH, ALB, ALT, bile acids, liver wts (a/r), RE hyperplasia and decreased HCT; females - increased MET, Seg neutro, RE hyperplasia. 4.5 ppm - Males - decreased HCT, HGB, RBC, and increased MET, SDH, ALB, ALT, AP, BA, liver wt (a/r), RE hyperplasia and kidney cytoplasmic pigmentation; females - decreased HCT, HGB, RBC, and inc. MET, Seg neut, ALB, AP, liver wt (a/r) and spleen wt (a/r) and RE hyperplasia. 9 ppm - Males - decreased HCT, HGB, RBC, Plat, and increased in RETIC, Nucl. RBC, MET, SDH, ALB, ALT, AP and BA, kidney wt (r) and liver wt (a/r), liver basophilia, kidney pigmentation and RE hyperplasia; females - decreased HCT, HGB, RBC, MCH, MCHC, PLAT, and increased RETIC, MET, SDH, SLB, AP, liver wt (a/r), spleen wt (a/r), and liver basophilia, kidney pigmentation, and RE hyperplasia. 18 ppm - Males - decreased HCT, HGB, RBC, PLAT, and increased RETIC, Nucl RBC, MET, SDH, ALB, ALT,AP, BA, kidney wt (r), liver wt (a/r), splenic wt (a/r), liver basophilia, kidney pigmentation, splenic congestion and RE hyperplasia; Females - decreased HCT, HGB, RBC, MCV, MCH, MCHC, PLAT and increased RETICS, MET, Nuc. RBC, SDH, ALB, ALT, AP, kidney wt (a/r), liver wt (a/r), splenic wt (a/r), liver basophilia, kidney pigmentation, splenic congestion, and RE hyperplasia.
No deaths were observed in this study nor were there treatment-related effects seen in body weight gain. No clear clinical signs of toxicity were observed. Hematology findings were found consistently in ONCB-treated groups and was consistent with methemoglobinemia and normocytic, normachromic anemia by the end of the study. Target organs identified in this study included the liver, spleen, kidney and nasal cavity (dorsal meatus and most anterior turbinate). Based on microscopic findings in the respiratory epithelium and methemoglobinemia seen at 1.1 ppm, a NOEL was not established in this study.

Test substance : Commerical grade ONCB analyzed confirmed purity > 99%.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
02.12.2002 (10)

Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 6 hr/day
Frequency of treatment : 5 days per week for 4 weeks
Post obs. period : none
Doses : 0, 10, 30, or 60 mg/m3
Control group : yes
NOAEL : = 10 mg/m³
Method : OECD Guide-line 412 "Repeated Dose Inhalation Toxicity: 28-day or 14-day Study"
Year : 1982
GLP : yes
Test substance : other TS
Method : Groups of 15 male and 15 female SD rats were exposed via whole body in stainless steel and glass inhalation chambers to airborne concentations of 0, 10, 30 or 60 mg/m3 ONCB for 6 hr/d, 5 days/wk for 4 weeks. Concentrations of ONCB were determined at least 3X daily using UV spectrophotometry. Parameters monitored in this study included daily morbidity and mortality checks, weekly detailed clinical observations and body weights. Hematology parameters (HGB, RBC, HCT, RETIC, MET, clotting time, RBC morph. and total and differential leukocytes) and clinical chemistries (BUN, SGPT,AP, GLU, ALB, TPROT, GLOB, K, CL, CA, PHOS) were analyzed at days 0 and just prior to termination (MET also analyzed after 2 weeks of testing) for 10 rats/sex/group. Ophthalmoscopic exams were conducted on all rats prior to study start and at termination. Organ (brain,testes, heart, kidneys, liver, lungs, spleen) weights and weight

ratios were recorded at terminal sacrifice for all rats on test. Microscopic examination of over 40 tissues and organs was performed on 10 rats/sex from the high dose and controls and spleens from 10 male and 10 female mid and low dose animals. Gormori's stain was used to semiquantitate the degree of hemosiderosis. A Bartlett's test was performed on study data to determine the degree of equality of variance. Parametric procedures (Snedecor and Cochran followed by Dunnett's test) were used for parametric parameters. The Kruskal-Wallis test, followed by Dunn's Summed Rank test, were used for nonparametric parameter analysis. P< 0.05 was used in all cases.

Result : Cumulative chamber concentrations were 0, 9.9, 30 and 59 mg/m3; thus good correlation occurred between nominal and analytical values. No effects were seen in ocular toxicity, body weight gain, or clinical signs and no deaths occurred. Effects of treatment were limited to increases in methemoglobin (MET), anemia, organ weight changes (liver, kidney and spleen) and microscopic findings (extramedullary hematopoiesis and hemosiderosis) seen in the spleen. Following are the significant effects seen at each dose level - 10 mg/m3: Males - liver wt increase (rel. wt only), small non-stat. significant increase in MET; Females - nonstatistically significant increase MET. 30 mg/m3 - Males - increased MET, liver wt (a/r), Kidney wt (a/r), spleen wt (a/r), small increase in splenic path.; Females - decreased RBC, HGB, HCT, and increased MET, liver wt (a/r), kidney wt (a/r) and splenic wt (a/r) plus slight spleen microscopy; 60 mg/m3 - Males - decreased RBC, HGB and increased MET, RET, liver, kidney and spleen wts (all a/r) and pathology of the spleen; Females - decreased RBC, HGB, HCT and increased HCT, MET, RET, and liver, kidney and spleen wts (a/r) and pathology of the spleen.

Test substance : Commercial grade ONCB with purity > 99%.

Reliability : (1) valid without restriction
Well documented study, conducted according to GLPs and meeting OECD guidance # 412; Supplemental information for HPV as a study of longer duration has been used to fulfill this endpoint.

06.12.2002 (13)

Species : rat
Sex : no data
Strain : other
Route of admin. : oral unspecified
Exposure period : up to 7 months
Frequency of treatment : daily
Post obs. period :
Doses : 70 mg/kg/d for 20 days; 5, 0.025, 0.005 and 0.0025 mg/kg/d for 7 months
Control group : yes
NOAEL : = .025 mg/kg bw
Method : other
Year : 1967
GLP : no
Test substance : other TS
Method : Peroral treatment of 20 albino rats at 70 mg/kg for 20 days, followed by peroral administration of ONCB to groups of rats at 5, 0.025, 0.005 and 0.0025 mg/kg/d for 7 months. Measured indices reportedly included hematology, liver function (blood and urine) and peripheral blood pathology.

Result : 0/20 rats treated with 70 mg/kg ONCB for 20 days died. Groups of rats treated for 7 months exhibited marked changes in peripheral blood. Methemoglobin levels were increased only during the seventh month of testing in the HD group; elevations occurred only at 5 mg/kg ONCB. Hemoglobin was reduced and reticulocytes, serum alkaline phosphate and urinary bilirubin were elevated along with presence of Heinz bodies in erythrocytes at 5 mg/kg/d. The NOEL was 0.025 mg/kg/d.

Conclusion : Comparative study using ONCB, MN CB and PNCB. Concluded that PNCB

was the most toxic isomer following systemic exposure, MNCB was intermediate, and ONCB was the least systemically toxic of the three isomers tested. All isomers exhibited essentially the same pattern of toxicity.

Reliability : (4) not assignable
Supplemental information, as this report provides but a summary of results without sufficient detail to be classified higher.

06.12.2002 (2)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : Salmonella typhimurium strains TA100, TA98, TA1535 and TA1537
Concentration : 0, 10, 33, 100, 333, 1000 ug/plate
Cycotoxic conc. : 1000 ug/plate
Metabolic activation : with and without
Result : positive
Method : OECD Guide-line 471 "Genetic Toxicology: Salmonella thyphimurium Reverse Mutation Assay"
Year : 1983
GLP : yes
Test substance : other TS
Method : Methodology used by NTP was based on Ames test plate incorporation assay and consistent with OECD 471. All tests were run in duplicate and three plates were assayed at each dosage for each run both with and without metabolic activation. S9 obtained from male S-D rats injected with Arochlor 1254 (500 mg/ml) five days before they were killed; all tester strains obtained originally from B. Ames; the high dose was designed to produce toxicity (reduced background lawn or solubility limits). Sterile DMSO was used as the solvent; negative (solvent) and positive controls (2-aminoanthracene, 4-nitro-o-phenylenediamine, sodium azide and 9-aminoacridine) were used as appropriate to detect mutagenicity with or without metabolic activation in each of the 4 tester strains used. A positive response was detected if a reproducible dose-related increase (>2X) was seen in revertant colonies according to a model described by Margolin et al, 1981).
Result : Positive in strain TA100 only with metabolic activation; inactive in TA100 without activation and in other tester strains with or without rat S9.
Test substance : purity greater than 99%
Reliability : (1) valid without restriction
Consistent with OECD guideline 471 and conducted according to GLPs.
Flag : Critical study for SIDS endpoint
06.12.2002 (12)

Type : Cytogenetic assay
System of testing : Chinese Hamster Ovary Cell in vitro assay
Concentration : 50 - 500 ug/mL
Cycotoxic conc. :
Metabolic activation : with and without
Result : positive
Method : other
Year : 1987
GLP : yes
Test substance : other TS
Method : Study conducted according to NTP study design; testing involved 2 labs and included 2 tests (1 per lab) without S9 and three tests (repeat at one lab) with S9. The latter used SD male rat Arochlor 1254-induced liver homogenate. Cell cultures were handled to prevent photolysis of Brdu-substituted DNA. Each test consisted of concurrent solvent and positive controls and at least 3 dose levels. Cells were incubated in McCoy's 5A

		medium with test agent for 14 hours (ONCB was found to induce cell cycle delay, thus 18.5 hrs was used in one test), colcemid added and incubated for an additional 2 hrs and harvested and processed. 100 first-division metaphase cells (200 cells per dose group were used in follow up studies to address equivocal results) were scored blind from prepared slides for each dose level. Classes of aberrations were recorded and included simple, complex, and other abnormalities. Statistical analyses (Armitage trend test;Margolin multiple comparison test) were conducted on both the dose-response curve and individual dose points; significance was determined as $p<0.05$ for single data points and $p<0.015$ for trend.
Result	:	Both tests using ONCB wihout metabolic activation resulted in equivocal and then negative results; no. of aberrant cells in the solvent control (DMSO), and at 16, 50 and 160 ug/ml (100 metaphases used) were 2, 8, 8, and 11; confirmatory test used 0, 47, 101 and 216 ug/ml which resulted in no. of aberrant cells of 3, 2, 0, 2 (used 200 metaphase cells). The initial test with S9 resulted in a negative finding, as the no. of aberrant cells were 4(DMSO control), 6 (50 ug/ml), 6 (160 ug/ml) and 6 (500 ug/ml); Subsequent studies using top doses of 465 and 500 ug/ml yielded 22 and 23 aberrant cells, respectively, vs a control value of 3 and thus was considered weakly positive .
Conclusion	:	ONCB is considered to be a weakly positive clastogen in the CHO in vitro assay only with metabolic activation.
Reliability	:	(2) valid with restrictions Well documented, GLP study.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (11)

5.6 GENETIC TOXICITY 'IN VITRO'

5.7 CARCINOGENITY

5.8 TOXICITY TO REP RODUCTION

Type	:	other
Species	:	mouse
Sex	:	male/female
Strain	:	CD-1
Route of admin.	:	gavage
Exposure period	:	22 weeks
Frequency of treatment	:	daily for study duration
Premating exposure period		
Male	:	7 days
Female	:	7 days
Duration of test	:	7 days pretest, 98 days breeding and up to 5 weeks for littering,and then until last litter is weaned
Doses	:	0, 40, 80, or 160 mg/kg/day
Control group	:	yes
NOAEL Parental	:	= 160 mg/kg bw
NOAEL F1 Offspr.	:	= 160 mg/kg bw
Method	:	other
Year	:	1992
GLP	:	yes
Test substance	:	other TS
Method	:	Standard Continuous breeding protocol designed by NTP and published as Lamb, 1985. J. Amer. Coll. Toxicol. 4:163-171. Based on 2 week toxicity

test to establish dose levels, animals are individually housed for 7 days, then cohoused in breeding pairs for 98 days, and allowed to propagate. During this period the following indices are recorded: clinical signs of toxicity, mortality, parental body weight and average consumption of water during representative weeks, fertility (e.g. no. of pairs producing a litter/number of breeding pairs), the no. of litters per pair, the no. live pups/litter, % pups born alive, sex ratio of pups and pup body weights after birth. The last litter born during the holding period (5 weeks) following the breeding period was reared until weaning. Thereafter treatment of the F1 animals was initiated and these animals used for assessment of second generation fertility. For this phase, siblings were cohoused until sexual maturity, when 20 non-sibling males and females per treatment group were cohabitated for 7 days and then housed singly through delivery. Endpoints for this mating trial were the same as for the F0 generation. At termination of F0 and F1 generations, animals were necropsied and evaluations made for organ weights (livers, ovaries or testes and epididymides from 5 per group, control and HD), body weights, epididymal sperm motility, sperm morphology, sperm count and estrual cyclicity. Methemoglobin measurements and spleen weights were recorded for both the F0 and F1 generations . Proportional data were assessed statistically using the Armitage trend test , with each dose group compared to control using a chi-square analysis. Absolute body and organ weights were compared using Shirley's or Dunns test while dose related trends were identified by Jonckheere's test. Vaginal cytology was analyzed using an analysis of variance described by Morrison to test for simultaneous equality. A p value of <0.05 or <0.01 was used.

Result : In the final litter of the holding period following the continuous breeding phase, pup weight gain during suckling was lower in all 3 treated groups vs. control; at weaning pups in the 160 mg/kg group weighed 12% less than controls. All other fertility and reproductive parameters were unaffected. F1 animals had significantly lower body weights at weaning but were significantly heavier than controls at mating and termination and thus not considered toxicologically significant. Thus, no adverse effects in reproductive endpoints were observed for F1 generation animals. Nonreproductive toxicity was observed similarly in both F0 and F1 animals from the 160 and 80 mg/kg dose groups and included: increased spleen and liver weights and methemoglobinemia.

Test substance : Test material with analytically confirmed purity > 99%.

Conclusion : Report concluded that "ONCB does not appear to be a reproductive toxicant, even in the presence of systemic toxicity, in Swiss CD-1 mice"

Reliability : (2) valid with restrictions
Well documented study using a unique protocol designed to provide reproductive toxicity evaluations.

Flag : Critical study for SIDS endpoint
06.12.2002 (10)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	Gestation days 6 - 15
Frequency of treatment	:	Once daily during the exposure period
Duration of test	:	Study terminated on gestation day 21
Doses	:	0, 25, 75, 100 or 150 mg/kg/d
Control group	:	yes
NOAEL Maternalt.	:	>= 25 mg/kg bw
NOAEL Teratogen	:	>= 100 mg/kg bw

NOAEL Embryotoxicity	:	>= 100 mg/kg bw
NOAEL Fetotoxicity	:	>= 100 - mg/kg bw
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	1987
GLP	:	yes
Test substance	:	other TS
Method	:	Groups of 25 mated female SD rats were originally dosed with 25, 75 or 150 mg/kg ONCB on gestation days 6-15; a control group of 25 mated female rats exposed only to corn oil served as a concurrent control. Due to significant mortality seen in the 150 mg/kg test group, an additional study group was added and treated with 100 mg/kg ONCB. Body weights, detailed physical examinations and individual food consumption were recorded on gestation days 0, 6, 10, 13, 16, and 21. Complete necropsies and examinations of the uterine contents were performed on all females in the 100 mg/kg group and below at time of sacrifice. Full-term fetuses were examined externally, sexed, weighed and prepared for teratogenic evaluation. Approximately one-half of the fetuses in each litter were processed for either visceral soft-tissue evaluations using Wilson's techniques or were used for skeletal evaluations. For interval data (body wts, wt changes, reproductive data) Bartlett's test was used to determine equality of variance and AN OVA and Dunnett's test used for parametric data while the Kruskal-Wallis test and Summed Rank test were used for nonparametric data. For incidence data (i.e. mortality rates, % and incidence of variations and malformations) comparisons were made using the Chi-square contingency table and the 2X2 Fisher Exact test using the Bonferroni inequality estimate; linear trend was evaluated using the Armitage test. Comparisons were made using the litter as the comparative entity. Both p values of <0.05 and 0.01 were reported..
Result	:	Six rats died between gestation days 6-13 at 150 mg/kg; one animal each died at 75 and 100 mg/kg while no deaths occurred in the control or 25 mg/kg test group. Maternal weight gain in the 25 and 75 mg/kg groups was greater than that of the concurrent controls throughout the study. Females in the 100 mg/kg test group had a reduced weight gain throughout the treatment period. No significant clinical observations associated with toxicity were seen. Food consumption was similar in controls and 25 mg/kg animals; significant decreases in food consumption were observed at 75 and 100 mg/kg during gest. days 6-16. Early resorptions were significantly increased at 75 mg/kg, although a similar response was not observed in the 100 mg/kg dose group. Other reproductive parameters and fetal body weights in the ONCB treated groups were similar to their respective controls. No increase in external, soft tissue or skeletal malformations was observed up to 100 mg/kg. Animals from the 150 mg/kg group were omitted from reproductive assessment due to the large no. of deaths in this group. While an increase in cervical # 7 rib was noted in litters from the 25 and 75 mg/kg groups no such effect was observed in litters from dams treated with 100 mg/kg and thus was not considered attributable to treatment.
Test substance	:	Test article > 99% pure and mixed in corn oil for administration in daily volumes of 10 ml/kg.
Reliability	:	(1) valid without restriction Well conducted study meeting test and GLP guidance; Supplemental information as a Reproductive study has been used to fullfill this endpoint.

02.12.2002 (14)

5.10 OTHER RELEVANT INFORMATION

5.11 EXPERIENCE WITH HUMAN EXPOSURE

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(14) Solutia study no. ML-82-090, Teratology study in rats with orthonitrochlorobenzene [EPA document no. 8EHQ-0590-0973]

(15) Solutia study no. MO1983X079. Acute toxicity of o-nitrochlorobenzene to Daphnia magna. MIC Environmental Sciences Report ES-83-SS-6. St. Louis, MO. USA.

(16) Solutia study no. MO20020689, Final report on analytical chemistry investigations - 1971 Special studies. St Louis Research Report no. 3819.

(17) Solutia study no. Y-73-180. Toxicological investigation of o-nitrochlorobenzene [EPA document no. 88-920000376].

(18) US EPA, 2002. ECOSAR model, version 0.99f.

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7.1 END POINT SUMMARY

7.2 HAZARD SUMMARY

7.3 RISK ASSESSMENT

I U C L I D

Data Set

Existing Chemical	:	ID: 100-00-5
CAS No.	:	100-00-5
EINECS Name	:	1-chloro-4-nitrobenzene
EINECS No.	:	202-809-6
TSCA Name	:	Benzene, 1-chloro-4-nitro-
Molecular Formula	:	C6H4ClNO2

Producer Related Part

Company	:	Solutia Inc.
Creation date	:	04.04.2002

Substance Related Part

Company	:	Solutia Inc.
Creation date	:	04.04.2002
Memo	:	
Printing date	:	09.12.2002
Revision date	:	
Date of last Update	:	06.12.2002
Number of Pages	:	29
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

25.11.2002

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	:	= 83.4 - ° C
Sublimation	:	
Method	:	other
Year	:	1991
GLP	:	no data
Test substance	:	other TS
Method	:	not referenced
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Citation from a reputable, universally accepted reference guide; value cited in the PNCB HSDB (2002).
Flag	:	Critical study for SIDS endpoint

25.11.2002 (7)

2.2 BOILING POINT

Value	:	= 242 - ° C at 1013.25 hPa
Decomposition	:	
Method	:	other
Year	:	1991
GLP	:	no data
Test substance	:	other TS
Method	:	not reported
Remark	:	Listed as 242 deg. C @ 760 mm Hg.
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Citation from a reputable, universally accepted reference guide; value cited in the PNCB HSDB (2002).
Flag	:	Critical study for SIDS endpoint

25.11.2002 (7)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= .1253 - hPa at 20° C
Decomposition	:	
Method		other (measured)
Year	:	1973
GLP	:	no
Test substance	:	other TS
Remark	:	Reported as 0.094 mm Hg @ 20 deg. C.
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Cited as peer-reviewed in PNCB HSDB (2002).
Flag	:	Critical study for SIDS endpoint

25.11.2002 (1)

2.5 PARTITION COEFFICIENT

Log pow	:	= 2.39 - at ° C
Method		other (measured)
Year	:	1989
GLP	:	no data
Test substance	:	other TS
Method	:	Followed EPA methodology as defined in USEPA-600/4-79-032; Shake flask method using 6 replicates.
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Value derived from well accepted study design and consistent with other measured values reported in the literature (i.e. Hansch and Leo, 1995, SRC. Howard, 1990. Handbook of Environmental Fate and Exposure for Organic Chemicals. Lewis Pub.)
Flag	:	Critical study for SIDS endpoint

04.12.2002 (8)

2.6.1 WATER SOLUBILITY

Value	:	= 154 - mg/l at 25 ° C
Qualitative	:	
Pka	:	at 25 ° C
PH	:	- at and ° C
Method	:	other
Year	:	1995
GLP	:	no
Test substance	:	other TS
Method	:	Group contribution method for calculation allows for the estimation of water solubility based on the chemical structure of a given compound. Values assigned to substructural units (referred to as "fragments") are summed to give a final solubility for the entire compound. The fragment values used in this method were compiled from the KWB1 (Klopman, G, S Wang, and DM Balthasar. 1992. J. Chem. Inf. Comput. Sci. 32:474-482) and WYMW (Wakita, K, M Yoshimoto, S Miyamoto and H Watanabe. 1986. Chem. Pharm. Bull. 34:4663-4681) group contribution methods. Additionally, as this method traditionally models liquids better than solids, a melting point term was included to improve the values generated for compounds considered solids (i.e. melting point > 25 deg. C).
Result	:	The estimated water solubility (Sw) of PNCB was reported as log Sw [mol/l] = 3.01. Based on a molecular weight of 157.56 g/mol, the Sw=154 mg/L.
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Experimental value of 189.4 mg/l @ 25 deg C also reported in same reference; additional literature references cite values between 225-230 mg/l at 20 deg C (HSDB, 2002).
Flag	:	Critical study for SIDS endpoint

04.12.2002 (6)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	other
Light source	:	Xenon lamp
Light spect.	:	- nm
Rel. intensity	:	- based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	
Conc. of sens.	:	
Rate constant	:	cm3/(molecule*sec)
Degradation	:	- 98 % after 5 hour(s)
Deg. Product	:	yes
Method	:	other (measured)
Year	:	1979
GLP	:	no data
Test substance	:	other TS
Method	:	Photochemical reactivity assay where 1 mL of PNCB in n-hexane was put in 1 L reaction vessel, followed by substitution of n-hexane vapor with air or nitrogen free from nitrogen oxides. PNCB was deposited in the reaction vessel, which corresponded to 1000 ul gas if vaporized and was irradiated at 25-30 deg. C for 5 hr with the Xenon lamp (ozone-less type, Ushio co.).Disappearance of TS measured by HPLC. Reaction by-products measured by GC-MASS.
Result	:	Rate of disappearance was influenced by the intensity of light passing through either of two reaction vessels used in this experiment, i.e. pyrex and quartz. The rate of disappearance of PNCB in air free of nitrogen, when tested in pyrex and quartz vessels, respectively, was 4.1% and 96%. When PNCB was tested in nitrogen free of nitrogen oxides in pyrex and quartz vessels, respectively, disappearance rates were 7.1% and 98%. The single reaction by-product identified in air free from nitrogen oxides was 4-Chloro-2-nitrophenol while p-chlorophenol was the only by-product identified in nitrogen free from nitrogen oxides.
Test substance	:	Laboratory synthesized, purity no reported.
Reliability	:	(2) valid with restrictions This study supports the photodegradative capacity of PNCB.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (4)

Type	:	other
Light source	:	
Light spect.	:	- nm
Rel. intensity	:	- based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	1500000 molecule/cm3
Rate constant	:	.0000000000001714 cm3/(molecule*sec)
Degradation	:	50 - % after 62.4 day
Deg. Product	:	
Method	:	other (calculated)
Year	:	2002
GLP	:	no
Test substance	:	other TS
Method	:	Used AOPWIN, v. 1.90 from EPIWIN, Syracuse Research Corp.
Result	:	Vapor phase of PNCB is susceptible to reaction with photochemically-produced hydroxyl (OH) radicals. The 2nd order rate constant for reaction with hydroxyl radicals was calculated as 0.1714E-12 cm3(molecule*sec). Based on 1.5E6 OH molecules/cm3 and assuming 12 hours of sunlight per day, the estimated photo-oxidation half-life is 62.4 days (~1500 hrs).
Test substance	:	p-Nitrochlorobenzene

Reliability	:	(2) valid with restrictions Value obtained from EPA recommended estimation model.

06.12.2002 (3)

3.1.2 STABILITY IN WATER

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other
Air (level I)	:	9.52
Water (level I)	:	28.5
Soil (level I)	:	61.8
Biota (level II / III)	:	
Soil (level II / III)	:	.171
Method	:	other
Year	:	2002
Method	:	Estimation using measured values from this dossier were incorporated into EPIWIN from Syracuse Research Corp., a methodology based on Meylan, 1993 as adopted by MacKay et al. 1996. Second Soil entry included estimation in Sediments. Values employed were : Mo. Wt = 157.56, vapor pressure of 0.094 mm Hg. Log Kow of 2.39, a melting point of 83 deg. C, and water solubility of 154 mg/L. Half lifes for air, water, soil and sediment were included as 1500 hr, 900 hr, 900 hr, and 3600 hr, respectively; emissions loading was 1000 kg/hr for each medium.
Remark	:	Persistence Time was 506 hr.
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Estimated values based on model recommended by US EPA.
Flag	:	Critical study for SIDS endpoint

04.12.2002 (3)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	domestic sewage
Concentration	:	1mg/l related to Test substance 10mg/l related to Test substance
Contact time	:	
Degradation	:	34 - 66 % after 24 hour(s)
Result	:	
Deg. Product	:	
Method	:	other

Year	:	1973
GLP	:	no
Test substance	:	other TS
Method	:	Semi-Continuous Activated Sludge (SCAS) test conducted over 10-month period, in accordance with J Am Oil Chemists Society methods (JAOCS, 1965, 42:986 and JAOCS, 1965, 46:432). Inoculum was municipal waste treatment sludge. Feeding rate started at 1 mg/24-h and was raised in 1 mg increments to 5 mg over 28 days, and held at 5 mg/24-h for 4 months, then raised again to 10 mg/24-h. Twenty mL samples of mixed liquor (activated sludge + liquor) were taken 1 hr after each addition and at the end of the aeration cycle, via sidearm stopcock. The mixed liquor was extracted and analyzed via UV spectroscopy. Spike recovery experiments were 95.9 +/- 1.5%.
Result	:	Average disappearance rate, days 75-120 (5 mg feed level, high aeration rate) was 33.9 +/- 2.9% over a 24-h cycle; over the next 60 days (same parameters) the disappearance rate was 30.7 +/- 9.4% over a 24-h cycle; over the last two weeks (10 mg feed level, low aeration), disappearance rate averaged 65.7 +/- 14.4% per 24-h cycle.
Test substance	:	PNCB presumably as commercial grade with purity > 99%.
Reliability	:	(2) valid with restrictions Study conducted prior to codification of GLPs but considered well documented. Methodology used has subsequently been incorporated into a standardized international test guideline for this study type.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (11)

Type	:	aerobic
Inoculum	:	other
Contact time	:	56 day
Degradation	:	13.4 - % after 56 day
Result	:	
Deg. Product	:	
Method	:	other
Year	:	1973
GLP	:	no
Test substance	:	other TS
Method	:	River Die-Away Test (RDA). River water was obtained from the Mississippi River near St. Louis, MO, USA. Settled water (2 days) was added (250 mL) to 500 mL narrow-mouthed bottles. Distilled water controls (with test substance) were prepared similarly to assess sorption to glass and volatilization. PNCB was added in 5 uL volumes, prepared with 5% (w/v) ethanol. Bottles were sealed with foil-lined caps and stored at room temperature in the dark. A positive control (LAS Reference # 1 Dodecene-1) was prepared similarly and used to verify the biological activity. Periodically, chemical analyses were made by sacrificing a bottle with PNCB and a control. A 25 mL aliquot of hexane was injected into the bottle, the bottle vigorously shaken, and the phases allowed to separate. A portion of the hexane was collected, trasferred to a 2 mL cell, and the UV absorption determined. Recoveries of spiked samples for PNCB were 97.6%.
Result	:	Losses from the distilled water control were insignificant (PNCB concentration of 1.008 mg/L at day 0 and 0.996 mg/L at day 56). PNCB concentration at day 0 was 0.992 mg/L and dropped to 0.859 mg/L at day 56 (a 13.4% loss due to biodegradation in 56 days).
Test substance	:	PNCB presumably as commercial grade with purity > 99%.
Reliability	:	(2) valid with restrictions Supplemental information for this Biodegradation HPV endpoint.

06.12.2002 (11)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

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	:	no
NOEC	:	= 1.8 -
LC50	:	= 6 -
Method	:	other
Year	:	1980
GLP	:	yes
Test substance	:	other TS
Method	:	Employed EPA methodology 660/3-75-009. Ten fish (ave. weight of 0.97 g and mean length of 40 mm), obtained from Trout Lodge, McMillin, WA, USA were tested in one of 5 test concentrations for up to 96-h. PNCB was administered in an acetone solution at concentrations of 1, 1.8, 3.2, 5.6 and 10 mg/L plus untreated and solvent control. Antimycin A was used as a positive control. Temperature was maintained at 12 +/- 1 deg. C. Tests were conducted in soft reconstituted deionized water, supplemented with 48 mg NaHCO3, 30 mg CaSO4, 30 mg MgSO4 and 2 mg KCL per liter. Fish were unfed 48 hr prior to testing and through the experimental period. Tests were conducted in 20-L glass vessels containing 15-L of solution. Dissolved oxygen was monitored to ensure the concentration did not fall below 2 mg/L before the end of the test. Water quality parameters such as pH, ammonia, and temperature were measured; no significant changes were observed during the test for these parameters. Estimation of LC50 and 95%CI were determined using EPA statistical procedures (probit analysis).
Result	:	The 96-h LC50 (95%CL) = 6.0 (4.8-7.6) mg/L.; the 48-h LC50 (95%CL) = 7.5 (6.1-9.2) mg/L; the 24-h LC50 (95% CL) = 8.8 (no CL calc.) mg/L. No deaths were observed up to 3.2 mg/L through 96 hrs. At the 5.6 mg/L level the following % mortality was reported at 24, 48 and 96-h: 0%, 10%, 50%. At 10 mg/L, mortality reached 70%, 90%, and 90% at 24, 48 and 96-h. Toxicity as exhibited by surfacing was seen at concentrations of 3.2 mg/L and higher beginning 24 hr after treatment while loss of equilibrium also was seen at 10 mg/L at all three time points. Dissolved oxygen ranged between 9.2-7.1 mg/L, pH between 7.2-7.6 and total nitrogen (NH3) of <0.1 - 0.3 mg/L.
Test substance	:	PNCB with purity of > 99%.
Reliability	:	(2) valid with restrictions Well documented study which followed regulatory guidance for study conduct. LC50 value identical to that reported for guppies (Deneer et al. 1987. Aquat Toxicol 10:115) and similar to LC50 of 8.3 for bluegill sunfish (Solutia study no. AB-80-316)
Flag	:	Critical study for SIDS endpoint

06.12.2002 (12)

Type	:	other
Species	:	other
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	
LC50	:	= 50.2 -
Method	:	other
Year	:	2002
GLP	:	
Test substance	:	other TS
Method	:	An acute freshwater fish 96-h LC50 was calculated using ECOSAR, from

the US EPA. The SAR for esters was used. The structure was determined from the CAS RN, as stored in the accompanying database of SMILES notations within ECOSAR.

Test substance : p-Nitrochlorobenzene.
Reliability : (2) valid with restrictions
Supplementary information.

25.11.2002 (21)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : no
NOEC : = 3.2 -
EC50 : = 10 -
Method : other
Year : 1980
GLP : yes
Test substance : other TS
Method : Employed EPA methodology 660/3-75-009. Ten < 24-h old D. magna Straus (lab culture) were tested at 23 deg C in a series of three replicates per test concentration. PNCB in dimethyl formamide was tested at 6.25, 12.5, 25, 50 and 100 mg/L plus untreated control and solvent control. Morbidity and mortality were checked daily. Tests were conducted in 250-mL beakers containing 200 mL of solution. Well water from St Peter, MO, USA was used. Daphnids received no food 48-h prior to treatment. Water quality was measured to record dissolved oxygen, pH, alkalinity, hardness and temperature. Determination of EC50 and 95%CL were made using EPA statistical procedures (Steven, CE 1976. ASTM STP 634).
Result : 48-h EC50 (95% CL) = 11.1 mg/L (8.9-13.3); 24-h EC50 = 18.8 (16.9-21.1) mg/L. Dissolved oxygen ranged between 8.1-8.4 mg/L, pH was 7.0-8.1, alkalinity was 266-340 mg/L and hardness ranged between 226-318 mg/L. Temperature remained constant at 23 deg. C. The NOEC was < 6.25 mg/L. Per cent deaths seen at 24 and 48 hr respectively were : none in control or solvent control, 6.25 mg/L - 3%, 23%; 12.5 mg/L - 7%, 50%, 25 mg/L - 83%, 90%, 50 mg/L - 100% at both time points and at 100 mg/L - 100% deaths at both time points.
Test substance : PNCB with purity of > 99%.
Reliability : (2) valid with restrictions Well conducted study following regulatory accepted test guidelines. Solutia study (AB-80-317) using similar design and employing two replicates per dose resulted in 48-h EC50 of 10 (9-12) mg/L.
Well conducted study following regulatory accepted test guidelines. Solutia study (AB-80-317) using similar design and employing two replicates per dose resulted in 48-h EC50 of 10 (9-12) mg/L.
Flag : Critical study for SIDS endpoint
06.12.2002 (19)

Type : other
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring :
EC50 : = 55.3 -
Method : other
Year : 2002
GLP :
Test substance : other TS

Method	:	Calculated an acute Daphnid 48-h EC50 employing ECOSAR from US EPA. The SAR for esters was used. The structure was determined from the CAS RN, as stored in the accompanying database of SMILES notations within ECOSAR.
Test substance	:	p-Nitrochlrobenzene
Reliability	:	(2) valid with restrictions Supplementary information. Supplementary information.

25.11.2002 (21)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	biomass
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	
EC10	:	= 2.2 -
EC50	:	= 8 -
growth EC10	:	= 4.9 -
growth EC50	:	= 16 -
Method	:	other
Year	:	1986
GLP	:	no data
Test substance	:	other TS
Method	:	DIN 38412, Part 9 - The green alga S. subspicatus (Strain 8681 SAG) was used to conduct a modified cell multiplication inhibition test. A stock solution of the test substance was prepared in double-distilled water and diluted to prepare a series of test concentrations ranging from 0.80-100 mg/L. The test was conducted in capped 250 ml Erlenmeyer flasks. Eight (8) replicates of each concentration were tested. Flasks were inoculated with the cell suspension (cell concentration of 10E5 cells/ml in each flask), placed on a white surface, protected from sunlight, shaken daily, and exposed to constant artificial lighting. The temperature was maintained at 24 +/- 1 deg C. and the relative humidity was 50%. A control group (8 replicates) was tested concurrently. On each measurement day, 50 ml were collected from each of two flasks from each test concentration or the control. The extinction value of the monochromatic radiation (578 nm wavelength) of the cell suspension was determined for each test concentration and the control. Samples were collected and measurements were made at the beginning of the test and after 24 and 48 hrs. Biomass determination was based on measurement of optical density (turbidity). EC values were determined by regression analysis.
Result	:	Mean measured values of control group at 48 hrs were extinction value - 0.068; Biomass - 3.6 x 10E5 cells/ml. Results of the cell multiplication inhibition test of PNCB were: 48-h Biomass EC10 = 2.2 mg/L; 48-h Biomass EC50 = 8.0 mg/L. The 48-h average specific growth rate EC10 = 4.9 mg/l; 48-h average specific growth rate EC50 = 16 mg/L.
Test substance	:	pNCB, purity unspecified.
Reliability	:	(2) valid with restrictions Small deviations from standard study design, including shorter duration used (48 vs 72 h), and limited information presented on each test concentration at each measurement point.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (5)

Species	:	other algae
Endpoint	:	
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	

EC50	:	c = 35.3 -
Method	:	An acute green algal 96-h EC50 was calculated using ECOSAR, from the USEPA. The SAR for esters was used. The structure was determined from the CAS RN, as stored in the accompanying database of SMILES notations within ECOSAR.
Test substance	:	p-Nitrochlorobenzene.
Reliability	:	(2) valid with restrictions Supplementary information.

25.11.2002 (21)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

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

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	20
Vehicle	:	other
Value	:	530 - mg/kg bw
Method	:	other
Year	:	1975
GLP	:	no
Test substance	:	other TS
Method	:	Methodology similar to OECD # 401, except with fewer animals; PNCB was administered by gavage in 10% corn oil to groups of 5 mixed sex SD rats at dosages of 398, 501, 631 and 794 mg/kg. Animals were observed for signs of toxicity and death daily for 14 days. Body weights were recorded on study day 0 and weekly thereafter. Animals dying and all survivors to d14 were necropsied. Food and water were given ad libitum and temp., humidity and light were controlled. LD50 and CI were calculated by the method of deBeer, J. Pharmacol Experiment Ther 86:1.
Result	:	LD50=530 mg/kg with CI of 480-590 mg/kg; Incidence of deaths observed at each dose group were: 1/5 @398 mg/kg, 2/5 @ 501 mg/kg, 4/5 @ 631 mg/kg, and 5/5 @ 794 mg/kg. Deaths occurred during study days 1-5, with most occurring during days 1-3. Clinical signs of toxicity observed included: increased weakness, slight tremors, ocular discharge. Necropsy of the viscera in decedents resulted in identification of lung hyperemia and discoloration of the liver, spleen and kidneys. Viscera of survivors (14 days) appeared normal.
Test substance	:	No data; assumed to be commercial grade with purity > 99%.
Reliability	:	(2) valid with restrictions Study conducted prior to codification of OECD guideline 401 or inception of US GLPs (1979). Fewer animals used than stipulated in 401. Test results are highly consistent with 17 other rat OLD50 values ranging betwen 294-830 mg/kg as found in the ECB IUCLID PNCB, 2000.
Flag	:	Critical study for SIDS endpoint

06.12.2002 (20)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	inhalation
Exposure period	:	6 hr/day
Frequency of treatment	:	5 days per week for 13 weeks
Post obs. period	:	
Doses	:	0, 1.5, 3, 6, 12 and 24 ppm
Control group	:	yes
NOAEL	:	< 1.5 - ppm
LOAEL	:	= 1.5 - ppm
Method	:	OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year	:	1989
GLP	:	yes
Test substance	:	other TS
Method	:	Groups of 10 male and 10 female F-344 rats were exposed in whole body stainless steel and glass chambers to vapors containing 0, 1.5, 3, 6, 12 or 24 ppm PNCB for 6 hr/d, 5 days per week, for 13 weeks. Vapor was generated by transfer of bulk PNCB into a flask and attached to a vapor generator with a rotary evaporation system. The resulting vapor was forced into a condenser and temperature maintained by circulating oil. Generator output and flow were automatically controlled. Chamber monitoring was performed using a GC/EC system. Low volatility of PNCB limited the maximum exposure vapor concentrations to the top level used in this study. Animals were individually caged, food and water administered ad libitum, and a 12 hr light:dark cycle employed. All animals were assessed for morbidity and mortality daily and weekly examined for clinical toxicity and recording of body weights. At termination of the study (13 weeks) all animals were necropsied and a full set of over 40 tissues and organs were examined microscopically for all high dose and control animals; target organs were examined for animals from lower dose groups. Organ weights and relative weights were assessed for all animals after 13 weeks of testing and included the following organs: heart, kidney, lung, liver, spleen, testis and thymus. The following hematology parameters were assessed on study day 1 (Methemoglobin only), 4, 23, and at 13 weeks from all rats from each study group: HCT, HGB, RBC, RETIC, MCV, MCH, MCHC, PLAT, WBC, MET, and WBC differentials. Similary, the following clinical chemistry parameters were measured from all rats at similar time points as hematology: BUN, CREAT, TPROT, ALB, GLOB, ALT, AP, CK, SDH, and bile acids. Williams parametric multiple comparison procedure was employed to statistically assess group-wise comparison of organ and body weights. Shirley's test for nonparametric analysis was used for clinical chemistry and hematology assessments. P<0.05 and <0.01 were used in all cases.
Remark	:	Sperm morphology and vaginal cytology evaluations were performed on rats exposed to 0, 6, 12, or 24 ppm PNCB. Male rats exposed to 24 ppm exhibited significantly lower left epididymal, cauda epididymal, and testis weights and lower spermatid heads/testis, spermatid counts and spermatozoal concentrations than control rats; estrous cycle length was decreased in all groups of PNCB-exposed females.
Result	:	Mean concentrations in all test chambers were between 99-100% of target concentrations. No treatment related deaths, obvious clinical signs of toxicity, or effects on body weight were observed at any dose level. Hematology findings were consistent with methemoglobinemia and macrocytic (increased MCV) and hyperchromic (MCHC increase) hemolytic anemia seen at all test levels. Compensatory hematopoietic cell proliferation was present and considerable hemosiderin deposition observed microscopically, and produced a pattern of effects observed with

other MET-forming agents. Following are the various statistically elevated/depressed effects noted at each dose level: At 1.5 ppm = increased MET, normocytic RBC (F only) and decreases in HCT, HGB, RBC, ALT (M only), renal hyaline droplet formation (males only), splenic congestion and hemosiderosis; at 3 ppm = increas ed MET, RETIC, normocytic RBC and bile acids (M only), and decreases in HCT, HGB, RBC, ALT, (M only), AP (F only), marked increase in spleen wt and mild liver wt (F only), renal hyaline droplets (M only), bone marrow hematopoietic cell proliferaton, Hardar ian gland inflammation, congestion and hemosiderosis of the spleen along with hematopoietic cell proliferation and capsular fibrosis and hemosiderosis of the liver Kupfer cells (F only); at 6 ppm = increases in RETIC, MET, n-RBC, bile acids (M only), SDH (F only), MVC, spleen and liver weights and decreases in HCT,RBC, ALT, AP, TPROT, GLOB, and renal hyaline droplet formation (M only), bone marrow and splenic hematopoietic cell proliferation, Hardarian gland inflammation, splenic congestion, hemosiderosis and capsular fibrosis of the liver; at 12 ppm = decreases in HCT, HGB, RBC,AP, GLOB, ALT, TPROT and increases in MET, RETIC, n-RBC, SDH and bile acids, marked increases in spleen weights and mild increases in liver, heart and thymus weights, renal hyaline droplet formation (M only), bone marrow and splenic hematopoietic cell proliferation, Hardarian gland cell proliferaton, splenic congestion, hemosiderosis, and capsular fibrosis and hemosiderosis and histiocytic hyperplasia of the liver; at 24 ppm = increases in MET, RETIC, MCV, n-RBC, HGB, SDH, bile acids and decreases in HCT, HGB, RBC, AP, GLOB, ALT, TPROT, organ weight increases of the spleen, liver, heart, thymus and decreased testes weight, renal hyaline droplet formation (M only), bone marrow and splenic hematopoietic cell proliferation, Hardarian cell proliferation, splenic congestion and capsular fibrosis, hemosiderosis of the spleen and liver, histiocytic liver hyperplasia and testicular atrophy

Test substance : PNCB determined to be > 97 % pure.
Reliability : (1) valid without restriction
Well documented study consistent with OECD test guideline 413
Flag : Critical study for SIDS endpoint
06.12.2002 (10)

Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 6 hr/day
Frequency of treatment : 5 days/week for 4 weeks
Post obs. period :
Doses : 0, 5, 15, and 45 mg/m3 (equivalent to 0.78, 2.3 and 7 ppm)
Control group : yes
NOAEL : < 5 - mg/m³
LOAEL : = 5 - mg/m³
Method : OECD Guide-line 412 "Repeated Dose Inhalation Toxicity: 28-day or 14-day Study"
Year : 1982
GLP : yes
Test substance : other TS
Method : Groups of 10 male and 10 female SD rats were exposed via whole body in stainless steel and glass inhalation chambers to airborne concentrations of 0, 5, 15 or 45 mg/m3 PNCB for 6 hr/day, 5 days/week for 4 weeks. PNCB was mixed with a solvent and fed into a spray atomizer through which dry air was passed. Test material flow into test chambers was controlled using a fluid metering pump. Concentrations of PNCB were determined at least 3X daily using UV spectrophotometer; particle size distribution was determin ed throughout the study. Parameters monitored in this study included daily morbidity and mortality checks, weekly detailed clinical observations, and body weights. Hematology parameters (HGB, RBC,

HCT, RETIC, MET, clotting time, RBC morph. and total and differential leukocytes) and clinical chemistries (BUN, SGPT, AP, GLU, ALB, TPROT, GLOB, K, CL, CA, PHOS) were analyzed at day 0 and just prior to termination (MET also analyzed after 2 weeks of testing) for 10 rats/sex/group. Ophthalmoscopic exams were conducted on all rats prior to study start and at termination. Organ (brain, testes, ovaries, heart, kidneys, pituitary, liver, lungs, spleen) weights and weight ratios were recorded at terminal sacrifice for all rats on test. Microscopic examination of over 40 tissues and organs were performed on all rats from the high dose and control groups at the end of the study. Spleens of all low and mid dose animals were also examined microscopically. Gormori's stain was used to semiquantitate the degree of hemosideros is. A Bartletts' test was performed on study data to determine the degree of equality of variances (Snedecor and Cochran) followed by Dunnet's test for parametric parameters and the Kruskal-Wallis test along with Dunn's Summed Rank test for nonparametric p arameter analysis. P <0.05 was used in all cases.

Result : Cumulative mean analytical exposure concentrations were 5, 16 and 45 mg/m3. Particle size distribution of the generated atmospheres established that PNCB was introduced as a vapor, rather than as an aerosol. No mortalities were observed in treated groups and mean body weights of PNCB-treated animals were similar to control values. Clinical signs of toxicity observed included: cyanosis of the conjuctivae, nasal areas and entire body in all three groups, with incidence increasing with dose. Other than a dark red appearance, no ocular abnormalities related to treatment were observed. Rats at all test levels exhibited slight reductions in HGB, HCT, RBC at one or both study intervals. Animals in the mid and high dose groups also exhibited an increase in the incidence of poikilocytosis and polychromia at the interim bleeding. MET showed a dose-related increase with levels approximating 2-8X controls. An increase in leukocytes was attributed to the abberant inclusion of reticulocytes in the automatic counting procedure for white blood cells. Small increases in GLU and reduced PHOS levels were seen in HD females only. Statistically elevated spleen and liver weights were seen in HD males and females (and rel. liver wts in mid dose females). An increased incidence of congestion, extramedullary hematopoiesis and hemosiderosis of the spleen was observed in male and female rats exposed to 45 mg/m3 and iron-positive pigmentation (hemosiderosis) in spleens of rats from the mid and low dose.

Test substance : Greater than > 99 % pure

Reliability : (1) valid without restriction
Provided as Supplemental information as a longer-term study, conducted by the same exposure route, has been selected as the Key study for this HPV Endpoint; this study meets OECD Test Guidance 412 and was conducted under GLPs.

06.12.2002 (17)

Species : rat
Sex : no data
Strain : other
Route of admin. : oral unspecified
Exposure period : up to 7 months
Frequency of treatment : daily
Post obs. period :
Doses : 110 mg/kg/d for 20 days; 5, 0.025, 0.005 and 0.0025 mg/kg/d for 7 months
Control group : yes
NOAEL : = .0025 - mg/kg bw
Method : other
Year : 1967
GLP : no data
Test substance : other TS
Method : Peroral treatment of 20 albino rats at 110 mg/kg for 20 days, followed by peroral administration of PNCB to groups of rats at 5, 0.025, 0.005 and

0.0025 mg/kg/d for 7 months. Measured indices reportedly included hematology, liver function (blood and urine) and peripheral blood pathology.

Result : 7/20 rats treated with 110 mg/kg PNCB for 20 days died. Groups of rats treated for 7 months exhibited marked changes in peripheral blood. Methemoglobin levels were increased within the first month of testing in the HD group; elevations occurred in groups treated with 0.005 mg/kg PNCB or higher. Hemoglobin was reduced and reticulocytes, serum alkaline phosphate and urinary bilirubin were elevated along with presence of Heinz bodies in erythrocytes at dosages of 0.005 mg/kg/d and above. The NOEL was 0.0025 mg/kg/d.

Conclusion : Comparative study using ONCB, MNCB and PNCB. Concluded that PNCB was the most toxic isomer following systemic exposure, MNCB was intermediate, and ONCB was the least systemically toxic of the three isomers tested. All isomers exhibited essentially the same pattern of toxicity.

Reliability : (4) not assignable
Supplemental information, as this report provides but a summary of results without sufficient detail to be classified higher.

06.12.2002 (2)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : Salmonella typhimurium strains TA100, TA98, TA1535, TA1537
Concentration : 10, 4, 3, 1.5, 1.3, 1, 0.3, 0.2, 0.04, and 0.01 mg/plate
Cycotoxic conc. : 3 mg/plate
Metabolic activation : with and without
Result : positive
Method : OECD Guide-line 471 "Genetic Toxicology: Salmonella thyphimurium Reverse Mutation Assay"
Year : 1979
GLP : yes
Test substance : other TS
Method : Method used was plate incorporation assay based on Ames test methods consistent with OECD 471. All tests were run in duplicate and three plates were assayed at each dosage for each run both with and without metabolic activation. The S-9 liver homogenates were prepared from male SD rats and CD-1 mice given Arochlor 1254. All tester strains were obtained from Dr. B. Ames. Sterile DMSO was used as the solvent and a solvent control was employed of 20 uL/plate DMSO. Positive controls used were: 2-aminoanthracene, 9-aminoacridine, benzo(a)pyrene, NaNo2 and 2-nitrofluorene. A positive response was determined upon observation of a statistically significant dose-response increase in revertant colonies. Bartlett's test was used for pairwise comparison to controls and dose response determined using regression analysis for log-log straight lines; P<0.01 was used.
Result : A definitive positive response was observed with TA1535 without metabolic activation, with some indication that TA1535 with metabolic activation was marginally positive.
Test substance : Greater than 99% pure
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
06.12.2002 (18)

Type : Cytogenetic assay
System of testing : Chinese Hamster Ovary Cell in vitro assay
Concentration : 50 to 5000 ug/mL
Cycotoxic conc. :
Metabolic activation : with and without

Result	:	positive
Method	:	other
Year	:	1987
GLP	:	yes
Test substance	:	other TS
Method	:	Study conducted according to NTP study design, testing involved 3 separate tests (2 with S9 and 3 without S9 fraction added) SD male rat Arochlor 1254-induced liver homogenate was used. Cell cultures were handled to prevent photolysis of Brdu-substituted DNA. Each test consisted of concurrent solvent and positive controls and at least 3 dose levels. Cells were incubated in McCoy's 5A medium with test agent ranging between 10.5-19 hrs, colcemid added and incubated for an additional 2 hrs and harvested/processed. 100 first-division metaphase cells were scored blind from prepared slides for each dose level. Classes of aberrations were recorded and included simple, complex and other abnormalities. Statistical analysis (Armitage trend test; Margolin multiple comparison test) were conducted on both the dose-response curve and individual dose points; significance was determined as P<0.05 for single data points and P<0.015 for trend.
Result	:	Initial trials run at harvest times of 10.5 hr w and w/o S9 were negative. A follow up trial w/o S9 conducted at a higher dose level (700, 800 and 900 ug/ml) and incubated for 19 hrs (because PNCB induced cell cycle delay in the earlier study) resulted in an increase in aberrant cells only at 900 ug/plate; A repeat of thi s study at levels of 500, 600 and 700 ug/plate resulted in a dose related increase only at the top dose used. Repeat of the metabolic activation trial using a longer period to harvest (19 hr) produced an increase in aberrant cells.
Reliability	:	(2) valid with restrictions Provided as Supplemental information as an in vivo cytogenetics study has been used to fulfill this HPV endpoint.

07.11.2002 (9)

5.6 GENETIC TOXICITY 'IN VITRO'

Type	:	Cytogenetic assay
Species	:	rat
Sex	:	
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	once
Doses	:	30, 100 and 300 mg/kg
Result	:	negative
Method	:	OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"
Year	:	1983
GLP	:	yes
Test substance	:	other TS
Method	:	Dose levels selected based on pilot study which produced 1/4 deaths @ 400 mg/kg and 4/4 deaths @ 600 mg/kg. Five rats/sex/time period were administered PNCB in corn oil by gavage. Metaphase cells were collected from rat bone marrow (femur) at harvest times of 6, 12 and 24 hrs after treatment from 5 rats/sex. Colchicine was administered 2 hr prior to sacrifice to arrest cells in c-metaphase. Marrow was exposed to hypotonic solution and fixed, cells and slides prepared and stained. All slides were coded before reading. Positive (cyclophosphamide) and negative (corn oil) controls were used for comparative purposes. Mitotic index was calculated based on counting of at least 500 slides and all breaks, deletions, translocations and other changes recorded. Breaks or aberrations between treated vs control groups were compared by Chi-square analysis. P <0.05 was used.

Result	:	Rats dosed with 100 and 300 mg/kg PNCB exhibited signs of cyanosis; animals given 300 mg/kg lost weight between time of dosing and sacrifice. No significant differences were observed in the frequency of breaks or aberrations between PNCB-treated and control groups at any of the three time points measured.
Test substance	:	Test material purity of > 99%.
Reliability	:	(2) valid with restrictions Time points measured did not include a period beyond 24 hr, but sufficient cells in metaphase were obtained at this time point that it was determined that there was no need to extend the sampling period.
Flag	:	Critical study for SIDS endpoint

04.12.2002 (13)

5.7 CARCINOGENITY

5.8 TOXICITY TO REP RODUCTION

Type	:	Two generation study
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	F0 & F1 Adults-premating through litter weaning (Fo) and postweaning (F1)
Frequency of treatment	:	daily (7d/wk) gavage
Premating exposure period		
Male	:	FO- 14 weeks; F!- 18 weeks
Female	:	FO-14 weeks; F1 - 18 weeks
Duration of test	:	FO M/F - 167d; F1 M/F- 219d
Doses	:	0, 0.1, 0.7 and 5.0 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL Parental	:	= 5 - mg/kg bw
NOAEL F1 Offspr.	:	= 5 - mg/kg bw
NOAEL F2 Offspr.	:	= 5 - mg/kg bw
Method	:	OECD Guide-line 416 "Two-generation Reproduction Toxicity Study"
Year	:	1984
GLP	:	yes
Test substance	:	other TS
Method	:	Test material was administered to groups of 15M and 30F rats (vehicle control group also included) in corn oil to F0 and F1 generations during a premating (14 wks for F0 and 18 wks for F1) growth period, and through the ensuing mating, gestation and lactation intervals (1 litter/generation). F1 rats continued on treatment during a post-weaning period of 30d. Dosing concentrations analyzed by GC weekly for the first week of the study and monthly thereafter for accuracy Body weights were recorded weekly for F0 and F1M. For F0 and F1 F wts were recorded weekly through the growth period and up to mating, then resumed after mating until sacrifice. Food consumption was recorded weekly for F0 and F1 M from study start up to mating, then resumed after mating through study term. Food consumption for adult females F0 and F1 was recorded weekly through the growth period and again after weaning of litters. Cageside observations for morbidity and mortality were made weekly, as well as daily observations of clinical signs. Temperature, humidity and light-dark cycles were controlled. F0 adults were sacrificed following weaning of the F1 litters and given a gross postmortem examination; reproductive tissues (testes, epididymides, seminal vesicles) were evaluated histopathologically for all control and high dose males. Adult F1 M and F rats were sacrificed

following completion of a post-weaning treatment interval, given a gross necropsy. A full histopathological examination of over 40 tissues and organs (including gonads) was performed on 10 randomly selected F1 adult animals/sex/group. Pups delivered to F0 and F1 females were evaluated for growth, survival and external irregularities during lactation days 0, 4, 14 and 21. F1 pups not selected for the adult generation were sacrificed and given a gross postmortem exam. Tissues were evaluated histopathologically (~40 tissues/organs) from 5/sex/group of F1 weanlings and F2 weanlings. Body weights and changes, food consumption, gestation length and number of offspring were analyzed using ANOVA techniques followed by Dunnet's Test for parametric parameters and Kruskal-Willis test followed by Dunn's Rank Sum for nonparametric analysis. Mortality and pregnancy rates, fetal and mating indices and pup survival were analyzed using Chi-square, followed by Fisher Exact test and Armitage's test for linear treand. The level of significance was reported at both the 5% and 1% levels.

Remark : Slight decreases in male and female fertility indices, as well as testicular effects seen in 3 HD male rats in the FO generation are considered spurrious findings, unrelated to treatment. No such effects were noted in the F1 generation, which were exposed over a considerably longer dosing period. Likewise, no testicular effects were observed in a group of 50 male rats exposed to 5 mg/kg/d PNCB by gavage for 24 months, a dosing regimen similarly used here, albeit for substantively longer than the 14 weeks rats in the HD group in this study were dosed.

Result : Dosing solutions were confirmed analytically as accurately prepared. No treatment-related mortalities could be affirmed in this study, although several gavage-related deaths occurred sporadically. Mean body weights and weight gains of all FO male groups were similar; FO females exhibited slightly, but not statistically lower, body weights at all treatment levels. This finding was considered unrelated to treatment as there was no dose-response effect noted. Food consumption values were similar between treated and control FO males and females, except for HD females which consumed slightly, but not statistically signficantly, more food through the first 6 weeks of the study. The mating index (no. mating/total given opportunity to mate) were similar for all FO Males. The mating index for FO Females was 86.7, 80, 71.4 and 71.4%, respectively, from control through HD group; as all these values were within the historical control range for this indice in this laboratory, these findings were considered unrelated to treatment. No statistical differences were seen in either pregnancy rate or male fertility index between PNCB-treated and control animals from the FO generation. Three HD male rats in the FO parental generation were found to have testicular degeneration upon microscopic examination. FO dams treated with PNCB during gestation and lactation exhibited mean body weights and length of gestation indices comparable to control levels. The number of live and dead pups at birth and pup weights durng lacation of pups from FO dams were unaffected by PNCB dosing. Pup survival in the HD group was slightly, but statistically significantly lower than the control group. This finding was related to the complete loss of two litters in this group, a phenomenon experienced within the test lab on an infrequent, but not unusual, basis. Thus, this finding was judged unrelated to PNCB treatment. No compound-related gross postmortem changes were observed in the FO adults or F1 weanlings. F1 generation: No treatment-related effects were seen in any test group for survival, mean body weights and gains, and food consumption during mating, gestation and lactation. No treatment-related effects were observed during the gross postmortem evaluation of F1 adults. An increase in extramedullary hematopoiesis and brown pigmentation of reticuloendothelial cells of both sexes of the HD treated groups were observed following histological examination of F1 animals.All three PNCB-treated groups of F1 females exhibited a slightly (but not statistically) lower mating index than the control group; however, no dose-response was evident and the number of pregnant females in all groups, control and treated, were similar. Thus, this

observation was not considered treatment-related. Male (FO1) mating and fertility indices were unaffected by treatment. Litter and pup survival indices in groups of F2 generation animals were comparable between all treated and control groups.Similarly, pup weights were unaffected by treatment at all test levels. No evidence of toxicity was observed during the gross postmortem evaluation of F2 pups and no compound-related changes were observed during histological examination of tissues in F1 and F2 weanling pups.

Test substance : ONCB with purity of 99.43%
Reliability : (1) valid without restriction
Well documented GLP study meeting OECD Test Guideline 416.
Flag : Critical study for SIDS endpoint
04.12.2002 (15)

Type : other
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 105 days
Frequency of treatment : daily, 7 days per week for 7 days prior to cohousing and 98 days of cohousing
Premating exposure period
Male : 7 days
Female : 7 days
Duration of test : 98 days of continuous breeding
Doses : 0, 62.5, 125 and 250 mg/kg/d
Control group : yes, concurrent vehicle
Method : other
Year : 1993
GLP : yes
Test substance : other TS
Method : Standard Continuous breeding protocol designed by NTP and published as Lamb, 1985. J. Amer. Coll. Toxicol. 4:163-171. Based on 2 week toxicity test to establish dose levels, animals are individually housed for 7 days, then cohoused in breeding pairs for 98 days, and allowed to propagate. During this period the following indices are recorded: clinical signs of toxicity, mortality, parental body weight and average consumption of water during representative weeks, fertility (e.g. no. of pairs producing a litter/number of breeding pairs), the no. of litters per pair, the no. live pups/litter, % pups born alive, sex ration of pups and pup body weights after birth). The last litter born during the holding period (5 weeks) following the breeding period is reared until weaning after which treatment of the F1 animals was initiated and these animals used for assessment of second generation fertility. For this phase, siblings were cohoused until sexual maturity, when 20 non-sibling males and females per treatment group were cohabitated for 7 days and then housed singly through delivery. Endpoints for this mating trial were the same as for the F0 generation. At termination of F0 and F1 generations, animals were necropsied and evaluations made for organ weights (ovaries or testes and epididymides from 5 per group, control and HD), body weights, epididymal sperm motility, sperm morphology, sperm count and estrual cyclicity. Methemoglobin measurements and spleen weights were recorded for both the F0 and F1 generations. Proportional data were assessed statistically using the Armitage trend test , with each dose group compared to control using a chi-square analysis. Absolute body and organ weights were compared using Shirley's or Dunns test while dose related trends were identi fied by Jonckheere's test. Vaginal cytology was analyzed using an analysis of variance described by Morrison to test for simultaneous equality. A p value of <0.05 or <0.01 was used.
Result : Fertility of mice dosed with PNCB decreased progressively with the

duration of dosing and with increasing dose and being statistically different from controls at the high dose level. Most mice exposed to 250 mg/kg were cyanotic. Spleen and liver weights of F1 PNCB-treated mice reportedly were significantly greater than those of the controls. Survival and body weights of F1 (final litter) and F2 pups were significantly decreased at 250 mg/kg and at 125 mg/kg (F1 only).

Test substance : pNCB with purity of 97%

Reliability : (1) valid without restriction
GLP study, peer reviewed and followed an established study design. Sumitted as Supplemental information as a previously listed rat 2-generation reproduction study fulfills this HPV endpoint.

06.12.2002 (10)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	once per day
Frequency of treatment	:	gestation days 6 through19
Duration of test	:	rats sacrificed on gestation day 20
Doses	:	5, 15 and 45 mg/kg/day
Control group	:	yes
NOAEL Maternalt.	:	< 5 - mg/kg bw
NOAEL Teratogen	:	= 15 - mg/kg bw
NOAEL Fetotoxicity	:	= 15 - mg/kg bw
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	1980
GLP	:	yes
Test substance	:	other TS

Method : Groups of 24 mated female rats were dosed daily by gavage (test material dissolved/suspended in corn oil) during gestation days 6-19. All rats were observed for mortality and abnormal behavior twice daily from gestation day 0 through day 20, at which time all animals were sacrificed and maternal spleen weights recorded. Detailed physical exams for signs of toxicity were recorded on study days 0, 6, 10, 15 and 20. Maternal body weights were recorded at several intervals throughout the study. At sacrifice the uterine horns were examined for implantation sites, resoptions and the number of viable or non-viable fetuses. The number of corpora lutea were also recorded. The sex and weights of all live fetuses were recorded and all fetuses were examined for external abnormalities. One-half of the fetuses per litter were examined for skeletal malformations while the other half were examined for internal anomalies. The following statistical analyses were performed: For interval data (body wts, wt changes, reproductive data) Bartlett's test was used to determine equality of variance and ANOVA and Dunnett's test used for parametric data while the Kruskal-Wallis test and Summed Rank test used for nonparametric data (Snedecor and Cochran). For Incidence data, i.e. mortality rates, % and incidence of variations and malformations comparisons were made using the Chi-square contingency table and the 2X2 Fisher Exact test employing the Bonferroni inequality estimate; linear trend was evaluated using the Armitage test. Comparisons were made using the litter as the comparative entity. Both the 5% and 10% level of statistical significance were reported for each parameter.

Result : Maternal toxicity (reduced body weight gain during the treatment period and increased spleen weights), fetotoxicity (increased no. resorptions/litter), embryotoxicity (increased no. fetuses with unossified sternebrae, incompletely ossified cervical and vertebral transverse

processes) and fetal skeletal (predominantly angulated ribs) malformations were observed at the 45 mg/kg dosage level. At 15 mg/kg, similar maternal toxicity but no fetotoxic/embryotoxic or teratogenic responses were observed. At 5 mg/kg, only a slight increase in spleen weight was observed in maternal animals.

Test substance : purity > 99%.
Conclusion : PNCB produced teratogenic effects only at dosages which produced significant maternal toxicity.
Reliability : (1) valid without restriction
Provided as Supplemental information as a 2-Generation Reproduction study has been used as the Key study to fulfill the Reproductive Toxicity HPV Endpoint. This study meets OECD Test Guideline 414 and was conducted under GLPs.

06.12.2002 (14)

Species : rabbit
Sex : female
Strain : New Zealand white
Route of admin. : gavage
Exposure period : gestation days 7 through 19
Frequency of treatment : once daily
Duration of test : animals sacrificed on gestation day 30
Doses : 0, 5, 15, 40 mg/kg
Control group : yes, concurrent vehicle
NOAEL Maternalt. : = 15 -
NOAEL Teratogen : = 15 -
Method : OECD Guide-line 414 "Teratogenicity"
Year : 1980
GLP : yes
Test substance : other TS
Method : Groups of 18 mated female NZ white rabbits were administered PNCB in corn oil (constant volume of 2 ml/kg) in corn oil at PNCB concentrations of 0 (vehicle control), 5, 15, and 40 mg/kg on gestation days 7-19. Animals were evaluated for detailed signs of toxicity on test days 0, 7, 10, 15, 19 and 30; body weights were recorded on test days 0, 7, 19 and 30. Daily observations were made for morbidity and mortality. Food and water were administered ad libitum and a 12 light:dark cycle was employed. Temperature and humidity were controled. All animals were examined externally and 1/2 were evaluated for soft tissue malformations and the other 1/2 for skeletal findings. The following statistical analyses were performed: For interval data (body wts, wt changes, reproductive data) Bartlett's test was used to determine equality of variance and ANOVA and Dunnett's test used for parametric data while the Kruskal-Wallis test and Summed Rank test used for nonparametric data (Snedecor and Cochran). For Incidence data, i.e. mortality rates, % and incidence of variations and malformations comparisons were made using the Chi-square contingency table and the 2X2 Fisher Exact test employing the Bonferroni inequality estimate; linear trend was evaluated using the Armitage test. Comparisons were made using the litter as the comparative entity. Both the 5% and 10% level of statistical significance were reported for each parameter.
Result : Mortality so high at 40 mg/kg level that this study group was terminated without additional data collection. 15 mg/kg and 5 mg/kg - no effects on survival or maternal body wts, no treatment-related effects in uterine implantation data, fetal wts or sexing data. No statistically significant differences seen in skeletal malformations between treated and control groups nor was there any treatment-related increase in the incidence of external or soft tissue findings.
Test substance : PNCB > 99% pure.
Reliability : (1) valid without restriction
Well conducted study following GLP guidance and OECD study design. Limited due to excessive no. of deaths at the high dose group which

disallowed any developmental toxicity information to be obtained from this study group. Supplemental information, as a 2-Generation Rat Reproduction study has been used to fulfill the Reproductive Toxicity HPV Endpoint

06.12.2002 (16)

5.10 OTHER RELEVANT INFORMATION

5.11 EXPERIENCE WITH HUMAN EXPOSURE

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(12) Solutia study no. AB-80-315. Acute Toxicity of p-Nitrochlorobenzene to Rainbow Trout.

(13) Solutia study no. BA-83-240. In vivo chromosomal aberration assay with p-Nitrochlorobenzene. [EPA Document No. 86940000673/TS-000055402].

(14) Solutia study no. BD-79-327. A Teratology Study with p-Nitrochlorobenzene in Rats.[also found in Nair et al. 1985. in Toxicity of Nitroaromatic Compounds, Hemisphere Publ.].[EPA Document no. 86940000663/TS-00055402]

(15) Solutia study no. BD-80-472. A Two Generation Reproduction Study of p-Nitrochlorobenzene in Rats. [EPA Document no. 86940000677/TS-00055406]

(16) Solutia study no. BD-80-530. A Teratogenicity Study in Rabbits with p-Nitrochlorobenzene. [EPA Document no. 86940000663].

(17) Solutia study no. BD-81-106. A Four Week Inhalation Toxicity Study of p-Nitrochlorobenzene in the Rat; [EPA Document no. FYI-OTS-1085-0455}[also found in Nair el al. 1986. Fundamen Appl Toxicol 6:618-627].

(18) Solutia study no. DA-79-258. Salmonella mutagenicity assay of CP 6560 [EPA Document no. 86940000672].

(19) Solutia study no. MO-83-X078. Acute Toxicity of Para-nitrochlorobenzene to Daphnia magna.

(20) Solutia study no. Y-75-48, Toxicological Investigation of p-Nitrochlorobenzene.

(21) USEPA ECOSAR model, v. 0.99f.

7.1 END POINT SUMMARY

7.2 HAZARD SUMMARY

7.3 RISK ASSESSMENT

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August 18, 2003

Marianne L. Horinko, Acting Adminstrator
U.S. Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Subject: Comments on the HPV Test Plan for the Category Chloronitrobenzenes

Dear Administrator Horinko:

The following comments on Solutia's test plan for the Chloronitrobenzenes category are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

Solutia, Inc. submitted its test plan on April 23, 2003 for the category Chloronitrobenzenes consisting of Benzene, 1-Chloro-2-Nitro- (CAS No. 88-73-3), Benzene, 1-Chloro-3-Nitro- (CAS No. 121-73-3) and Benzene, 1-Chloro-4-Nitro- (CAS No. 100-00-5), also known as ONCB, MNCB, and PNCB, respectively. All three members of the Chloronitrobenzenes category are manufactured in the U.S. by Solutia at a single site. Chloronitrobenzenes are then sold to a limited number of customers for the express purpose of full chemical conversion into other industrial chemicals that are ultimately used in the preparation of dyes and pigments, pesticides, animal feed ingredients, polymer additives, veterinary pharmaceuticals, and water-treatment chemicals. Solutia has submitted a comprehensive analysis of Chloronitrobenzenes by compiling substantial amounts of existing data from a variety of sources. In addition, this company combined three Chloronitrobenzene isomers with similar chemical, pharmacological, and toxicological properties into a single category for purposes of the HPV program. This approach demonstrates a thoughtful analysis by Solutia, in addition to being a scientifically valid analysis of a chemical's toxicity and adequate for a screening level program. Information from existing data or data derived from estimation models for physicochemical properties, environmental fate, and human and environmental effects of the three Chloronitrobenzene isomers have led Solutia to conclude that no additional testing is necessary under the HPV Challenge program.

Comparative investigations of the three Chloronitrobenzene isomers show a similar mode of action with the order of potency to be: para isomer > meta isomer >> ortho isomer. Toxicity information on PNCB, the most toxic of the three isomers, and ONCB is available for all SIDS endpoints in the HPV program. Some of the endpoints for MNCB have been appropriately filled using a "read-across" approach based on extensive information on para and ortho isomers. We commend Solutia's efforts in drawing on all available information from a myriad of sources to meet the SIDS endpoints for the chemical category Chloronitrobenzenes. This approach is consistent with the EPA's stated goals of maximizing the use of existing data in order to limit additional animal testing. We would also like to point out, as Solutia has in their test plan, that there are marked species differences in susceptibility to Chloronitrobenzene toxicity, i.e. their ability to form methemoglobin. Humans are decidedly more affected than rodent species and toxicity tests in rodents are NOT considered fully representative of the high acute toxicity to humans elicited by these chemicals. Furthermore, there is sufficient experience with occupational exposure in humans that a TLV has been established for PNCB. Additional testing in animals will not further protect humans, and the TLV is adequately protective.

We applaud Solutia on a well-written, thorough test plan for the category Chloronitrobenzenes and concur that no additional testing is needed for the purposes of the HPV program. Thank you for your attention to these comments. I may be reached at 202-686-2210, ext. 327, or via e-mail at meven@pcrm.org.

Sincerely,

Megha Even, M.S.
Research Analyst

Chad B. Sandusky, Ph.D.
Director of Research

201-14688

NCIC HPV
Sent by: Nguyet Phan

08/22/03 10:31 AM

To: NCIC HPV, moran.matthew@epa.gov
cc:
cc:
Subject: Environmental Defense comments on the Chloronitrobenzenes Category

Richard_Denison@environmentaldefense.org on 08/21/2003 03:43:34 PM

To: oppt.ncic@epamail.epa.gov, hpv.chemrtk@epamail.epa.gov, Rtk Chem/DC/USEPA/US@EPA, Karen Boswell/DC/USEPA/US@EPA, dalede@solutia.com
cc: lucierg@msn.com, kflorini@environmentaldefense.org, rdenison@environmentaldefense.org

Subject: Environmental Defense comments on the Chloronitrobenzenes Category

(Submitted via Internet 8/21/03 to oppt.ncic@epa.gov, hpv.chemrtk@epa.gov, boswell.karen@epa.gov, chem.rtk@epa.gov, lucierg@msn.com and dalede@solutia.com)

Environmental Defense appreciates this opportunity to submit comments on the robust summary/test plan for the Chloronitrobenzenes Category.

The test plan and robust summaries for the chloronitrobenzenes was prepared by Solutia, Inc. There are three chemicals in the proposed category: o-chloronitrobenzene (CAS # 88-73-3), m-chloronitrobenzene (CAS # 121-73-3) and p-chloronitrobenzene (CAS # 100-00-5). This is the last of a series of three test plans prepared by Solutia, Inc. on a series of related chemicals; the other two were the mononitroanilines and 4-nitrophenol. We commend the sponsor for resisting the temptation to lump the mononitroanilines and 4-nitrophenol together with the chloronitrobenzenes as a single category. We agree that the proposed chloronitrobenzene category is scientifically justified and we support it.

Considerable data exist for the three chloronitrobenzenes and the sponsor proposes to use limited read-across methods to fulfill the remaining required SIDS endpoints, hence maintaining that no additional studies are needed. We agree with the proposed test plan but we are concerned with some of the risk assessment statements made in the test plan as they relate to relative potency and margin of safety analysis. Specific comments are as follows:

1. According to the sponsor, the chloronitrobenzenes are manufactured by a single producer in the U.S. at a single manufacturing site in an essentially closed and continuous process. A TLV of 0.1 ppm has been established for p-chloronitrobenzene (PCNB) -- indicative of the significant toxicity of these chemicals. Allowable levels are 1.5 ppm for m-chloronitrobenzene (MCNB) and o-chloronitrobenzene (OCNB). The sponsor states that practices are in place to minimize worker exposure, but those practices have not been described. Also, since all proposed category members are expected to act similarly, levels of worker exposure to the three individual chloronitrobenzenes should be aggregated in assessing risks from such exposures.

2. The chloronitrobenzenes are important intermediates in the synthesis of

numerous industrial chemicals such as dyes, pigments, pesticides, veterinary pharmaceuticals and water treatment chemicals. The sponsor states that there are no known consumer uses of category members and that emissions are minimal. However, no information was provided on environmental releases (air or water) during the production of different products. It seems plausible that the emissions for some uses of the chloronitrobenzenes might be greater than it is for others. While some of these potential releases would likely come from the facilities of Solutia's customers for these chemicals, we encourage the sponsor to provide environmental release data if available for the different uses of the chloronitrobenzenes.

3. Many of the studies on ecotoxicity endpoints presented in the robust summaries were not conducted under GLP, but they do seem to be well done and read-across is not used to fulfill ecotoxicity endpoints, so we agree with the sponsor that no additional studies are needed.

4. The data provided for mammalian toxicity endpoints are adequate to support the proposed read-across to fulfill the repeat dose, reproductive and developmental endpoints for MCNB.

5. The sponsor states that PCNB is the most toxic of the chloronitrobenzenes; however, this statement is not supported by data presented in the robust summaries. For example, all proposed members induce methemoglobinemia and rat acute toxicity data show that MCNB is the most toxic, followed by OCNB and then PCNB. Moreover, inhalation repeat dose studies indicate no difference in the NOEL for OCNB and PCNB. These studies also show that OCNB induces epithelial hyperplasia of the respiratory tract at low doses, whereas PCNB does not cause this effect even at high doses. Mutagenicity studies indicate that OCNB and PCNB are equipotent. Therefore, the data indicate that PCNB cannot be considered the most toxic of the chloronitrobenzenes. Rather, in our view, rank ordering done for purposes of read-across needs to reflect the actual toxicity values for a given endpoint for the category members. It should also be assumed that any effect caused by any of the proposed category members will occur for all members.

6. The sponsor states that there is an adequate margin of safety for occupational exposures to the chloronitrobenzenes. This is a risk assessment statement and there is inadequate data presented in the test plan and robust summaries to justify it. We also note that inhalation repeat dose studies on OCNB indicate that hyperplasia of the respiratory epithelium and methemoglobinemia are occurring at a dose of 1.1 ppm -- an exposure level quite close to the TLV. This finding does not indicate an adequate margin of safety.

Thank you for this opportunity to comment.

George Lucier, Ph.D.
Consulting Toxicologist, Environmental Defense

Richard Denison, Ph.D.
Senior Scientist, Environmental Defense

August 26, 2003

Donald A. Lederer
Product Stewardship Manager
Solutia Inc
PO Box 66760
St. Louis, MO 63166-6760

Dear Mr. Lederer:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for the Chloronitrobenzenes Category posted on the ChemRTK HPV Challenge Program Web site on April 23, 2003. I commend Solutia, Inc. 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 Solutia advise the Agency, within 60 days of this posting on the Web site, of any modifications to its submission. Please send any electronic revisions or comments to the following addresses: oppt.ncic@epa.gov and chem.rtk@epa.gov.

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 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,

Oscar Hernandez, Director
Risk Assessment Division

Enclosure

cc: C. Auer
R. Gonzalez
W. Penberthy
M. E. Weber

EPA Comments on Chemical RTK HPV Challenge Submission:
Chloronitrobenzenes Category

Summary of EPA Comments

The sponsor, Solutia, Inc., submitted a test plan and robust summaries to EPA for the chloronitrobenzenes category dated April 9, 2003. EPA posted the submission on the ChemRTK HPV Challenge Web site on April 23, 2003. The category consists of three compounds: 1-chloro-2-nitrobenzene (CAS No. 88-73-3), 1-chloro-3-nitrobenzene (CAS No. 121-73-3), and 1-chloro-4-nitrobenzene (CAS No. 100-00-5).

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 is adequate.

2. Physicochemical Properties. The data provided by the submitter for melting point, boiling point, vapor pressure, and octanol/water partition coefficient are adequate for the purposes of the HPV Challenge Program. The submitter needs to provide measured water solubility data for at least one additional chemical.

3. Environmental Fate. The data provided by the submitter for these endpoints are adequate for the purposes of the HPV Challenge Program. Although EPA agrees that this chemical is stable in water, the submitter needs to explain this conclusion in the robust summary.

4. Health Effects. EPA reserves judgement on the adequacy of data pending verification of the test substances used in genetic toxicity studies and the correct name and purity of the test substance used in the reproduction toxicity study.

5. Ecological Effects. Adequate data exist to satisfy the fish and invertebrate endpoints for the purposes of the HPV Challenge Program. Although each of the algal studies is limited, when considered together, they are adequate for the purposes of the HPV Challenge Program.

EPA requests that the submitter advise the Agency within 60 days of any modifications to its submission.

EPA Comments on the Chloronitrobenzenes Challenge Submission

Category Definition

The submitter proposed a category to cover three isomers of monochlorinated nitrobenzenes: 1-chloro-2-nitrobenzene (ONCB, CAS No. 88-73-3), 1-chloro-3-nitrobenzene (MNCB, CAS No. 121-73-3), and 1-chloro-4-nitrobenzene (PNCB, CAS No. 100-00-5). The category definition is clear and unambiguous.

Category Justification

The submitter's justification of the chloronitrobenzenes category is based on similarities in the chemical structures of the three structural isomers of chloronitrobenzene, which are expected to result in "similar or identical properties" and "similar or identical biological mode[s] of action".

EPA agrees that he physicochemical and environmental fate properties of ONCB, MNCB, and PNCB are reasonably similar. In addition, the mammalian toxicity endpoints demonstrate comparable acute and chronic toxicities and similar primary toxic effects. Although the available acute aquatic toxicological data demonstrate some differences in the magnitude of toxicities of these compounds, the range in values is sufficiently limited to support the submitter's expectation for similar aquatic toxicological properties for the three isomers. Consequently, the data generally support the category.

Test Plan

Physicochemical Properties (melting point, boiling point, vapor pressure, partition coefficient and water solubility)

The data provided by the submitter for melting point, boiling point, vapor pressure, and octanol/ water partition coefficient are adequate for the purposes of the HPV Challenge Program.

Water solubility. The submitter provided an experimental value of 189.4 mg/L for PNCB, which is adequate for the purposes of the HPV Challenge program. However, the submitter provided calculated values for ONCB and MNCB. According to OECD guidelines, measured (experimental) values need to be provided unless the calculated values are less than 1 μg/L at 25 °C. Therefore, the submitter needs to provide a measured water solubility value for at least one of these chemicals (ONCB or MNCB) so the data can be read across to the third chemical. Ideally, measured data should be provided for the chemicals with the lowest and highest solubilities.

Environmental Fate (photodegradation, stability in water, biodegradation, fugacity)

The data provided by the submitter for these endpoints are adequate for the purposes of the HPV Challenge Program.

Stability in water. The test plan states that these chemicals are stable in water owing to a lack of hydrolyzable functional groups. This is not strictly correct, as the chlorine atoms in ONCB and PNCB are substantially more labile than in simple chlorobenzenes. However, EPA agrees that hydrolysis of the chlorine substituent is unlikely under normal environmental conditions. The submitter needs to explain its conclusion in robust summary format.

Biodegradation. The data provided by the submitter are adequate for the purposes of the HPV Challenge Program. The submitter needs to correct the description of biodegradation in the test plan (page 15), which states that the Semi-Continuous Activated Sludge (SCAS) tests "followed similar standards for conduct subsequently codified into OECD guideline 301". This type of test was codified into OECD Guideline 302A (modified SCAS test). EPA agrees with the submitter that these chemicals are not readily biodegradable (test plan, page 16).

Health Effects (acute toxicity, repeated-dose toxicity, genetic toxicity, and reproductive/developmental toxicity)

Pending verification of some information for genetic and reproductive toxicity, adequate health effects data were submitted for the chloronitrobenzenes category for the purposes of the HPV Challenge Program. Data for all category members were submitted for acute and genetic toxicity endpoints. In addition, data for the ONCB and PNCB were submitted for the repeated-dose, reproductive, and developmental toxicity endpoints. A read-across strategy for MNCB is acceptable for these endpoints.

Genetic Toxicity. EPA reserves judgement on the adequacy of the bacterial mutagenesis assays, pending receipt of robust summaries that identify the test compounds by name.

Reproductive Toxicity. EPA reserves judgement on the adequacy of this endpoint for ONCB, pending receipt of a revised robust summary that identifies the organs examined for histopathology, and for PNCB, verification of the compound name.

Ecological Effects (fish, invertebrates, and algae)

Adequate data exist to satisfy the endpoints for acute toxicity to fish and aquatic invertebrates for the chloronitrobenzenes category. When the experimental toxicity values reported from studies on algal toxicity are evaluated together, the data are adequate. The submitter should provide the missing data elements in the robust summary for each endpoint. See the specific comments on robust summaries (below) for details.

Algae. Considered separately, the 96-hour tests in *Chlorella pyrenoidosa* are insufficient due to the limited details available (especially the lack of a defined endpoint), and the 48-hour tests are inadequate because at least 72-hour tests are needed to satisfy the algal toxicity endpoint. However, the data are sufficient when considered together because (1) the experimental results and the ECOSAR predictions are similar, (2) the 96-hr studies were conducted according to OECD Guideline 201, and (3) additional studies located by EPA (Canton et al., 1985; Knie et al., 1983) show results similar to the submitted study results.

There were several inconsistencies in the test plan. First, Tables 1, 2, and 3 (pp. 11-13) reported that estimation methods were not available for aquatic toxicity endpoints for ONCB or PNCB or for acute fish toxicity for MNCB. Predicted toxicity values, however, were included in the test plan for each of the three isomers for all three aquatic toxicity endpoints. Second, page 17 reports that ECOSAR predictions were reported for daphnids and algae; however, predictions for fish were also reported. Finally, Table 6 (p. 17) indicates that a 48-hour algal EC_{50} was estimated for the MNCB. The estimated EC_{50} value, however, was a 96-hour value.

Specific Comments on the Robust Summaries

Generic Comments

The following comment applies to all the robust summaries provided by the submitter. The submitter should consult EPA guidance documents for the preparation of robust summaries (http://www.epa.gov/opptintr/chemrtk/guidocs.htm).

Each summary should clearly identify the test substance by the chemical name.

Health Effects

Some robust summaries did not identify the test substance at all and others identified the compound only by its acronym. The submitter needs to revise the summaries, especially for the studies that did not identify the test substance.

Genetic Toxicity. Robust summaries for mutagenesis assays in *Salmonella typhimurium* for the need to specify the test material.

Reproductive Toxicity. A robust summary for a continuous breeding assay in mice exposed to ONCB by gavage needs to identify the organs examined for histopathology and include separate NOAEL fields for systemic and reproductive toxicity.

The submitter needs to identify the test substance in the summary in the PNCB dossier for a two-generation reproductive toxicity assay in rats, which identified the chemical as ONCB under "Test substance" but as PNCB in the results section and PNCB in the reference list. The submitter needs to include separate NOAELs for systemic and reproductive toxicity.

Developmental Toxicity. The summary for the ONCB inappropriately used the ">=" symbol rather than the "=" symbol in the NOAEL fields for doses that were not the highest dose levels.

Ecological Effects

ECOSAR predictions were reported for each chloronitrobenzene isomer for all endpoints; however, no details on the inputs used to generate the predictions were reported. Also, the robust summaries indicated that the SAR for esters was used for all of the predictions although none of the sponsored chemicals are esters. From independent model runs, it appears that the submitter correctly used the SAR for neutral organics for toxicity predictions for MNCB. However, it is not clear how the submitter determined the predicted toxicity values for the other two isomers.

Fish. Important details missing from one or more summaries included results based on measured concentrations, values for the actual test concentrations, use and response of controls, mortality data, 95% confidence intervals, statistical methods, and concentration of the solvent (acetone).

Invertebrates. Important details missing from one or more robust summaries included test substance identity and purity, mortality data, and the concentration of the solvent.

Algae. Important details missing from one or more robust summaries included test substance purity, type of test (*e.g.*, static, semi-static, or flow-through), pH at the beginning and end of the test, water hardness, specific test concentrations (although ranges were provided), type of regression analysis used to determine the EC_{50} values, and which endpoint (biomass, etc.) was reported.

Followup Activity

EPA requests that the submitter advise the Agency within 60 days of any modifications to its submission.

References

Canton, J.H., et al. 1985. Toxicity, Biodegradability and Accumulation of a Number of Cl/N-Containing Compounds for Classification and Establishing Water Quality Criteria. Regul. Toxicol. Pharmacol. 5: 123-131.

Knie, J., et al. 1983. Results of Studies on Chemical Substances with Four Biotests. (Ergebnisse der Untersuchungen von Chemischen Stoffen mit Vier Biotests.) Dtsch. Gewaesserkd. Mitt. 27(3): 77-79.

201-14392A

Anh Nguyen
06/08/2004 07:27 AM

To: NCIC HPV@EPA
cc:
Subject: Fw: Response to the Comments on the HPV Submission for the Chloronitrobenzenes Category

----- Forwarded by Anh Nguyen/DC/USEPA/US on 06/08/2004 07:27 AM -----

"Lederer, Don A" <dalede@solutia.com>
06/07/2004 03:18 PM

To: NCIC OPPT@EPA, Rtk Chem@EPA
cc: "Downes, James E" <jedown@solutia.com>, Elmer Rauckman <rauckman@toxicsolutions.com>, J Downes <jdownes@charter.net>
Subject: Response to the Comments on the HPV Submission for the Chloronitrobenzenes Category

Attached are documents pertaining to Solutia's response to the comments of EPA and other organizations on the HPV Submission for the chloronitrobenzenes category.

Regards,
Don Lederer, CHMM
Product Stewardship Manager
Solutia Inc.
314/674-1113

RS-PCNB-Final.rtf NCB Response Outline Final.doc NCB Test Plan - Final.doc NCB Response Outline Final.doc

NCB Test Plan - Final.doc NCB Cover Letter Response to Comments.doc

SOLUTIA

Solutions for a better life.

201-15339

Don Lederer, CHMM
Product Steward
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575 Maryville Centre Drive
St. Louis, Missouri 63141

P.O. Box 66760
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Tel 314-674-1113
Fax 314-674-8808
dalede@Solutia.com

June 7, 2004

Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116
Attn: Chemical Right-to-Know Program

RE: **HPV Chemical Challenge Program**
Response to Comments
AR-201-14392
Chloronitrobenzenes Category
o-nitrochlorobenzene, CAS No. 88-73-3
m-nitrochlorobenzene, CAS No. 121-73-2
p-nitrochlorobenzene, CAS No. 100-00-5

We are pleased to provide the Agency our responses to comments received from EPA and other stakeholders on our referenced HPV Chemical Challenge submission for the Chloronitrobenzenes Category, which you will find attached. We are forwarding responses to the specific comments, along with a revised Test Plan and Robust Summary package.

Thank you for your consideration. Please contact me directly should there be any question related to this submission.

Sincerely,

Donald A. Lederer, CHMM
Product Stewardship Manager

Response to Comments on HPV Challenge Submission

Chloronitrobenzenes Category

CAS Number 88-73-3; 1-chloro-2-nitrobenzene

CAS Number 121-73-3; 1-chloro-3-nitrobenzene

CAS Number 100-00-5; 1-chloro-4-nitrobenzene

EPA Comments

Specific Comments on the Test Plan

COMMENT 1: *Water solubility*. The submitter provided an experimental value of 189.4 mg/L for PNCB, which is adequate for the purposes of the HPV Challenge program. However, the submitter provided calculated values for ONCB and MNCB. According to OECD guidelines, measured (experimental) values need to be provided unless the calculated values are less than 1 µg/L at 25°C. Therefore, the submitter needs to provide a measured water solubility value for at least one of these chemicals (ONCB or MNCB) so the data can be read across to the third chemical. Ideally, measured data should be provided for the chemicals with the lowest and highest solubilities.

RESPONSE: Measured values for the water solubility of all three isomers have been located and included in the Test Plan and new Robust Summaries have been prepared that use the estimation method as supporting evidence for experimental values**.**

COMMENT 2: *Stability in water*. The test plan states that these chemicals are stable in water owing to a lack of hydrolyzable functional groups. This is not strictly correct, as the chlorine atoms in ONCB and PNCB are substantially more labile than in simple chlorobenzenes. However, EPA agrees that hydrolysis of the chlorine substituent is unlikely under normal environmental conditions. The submitter needs to explain its conclusion in robust summary format.

RESPONSE: Robust summaries, based on chemical principles, for the water stability of all three isomers have been prepared and added to the robust summaries. The test plan text and tables have been updated to reflect the more explicit estimation method**.**

COMMENT 3: *Biodegradation*. The data provided by the submitter are adequate for the purposes of the HPV Challenge Program. The submitter needs to correct the description of biodegradation in the test plan (page 15), which states that the Semi-Continuous Activated Sludge (SCAS) tests "followed similar standards for conduct subsequently codified into OECD guideline 301". This type of test was codified into OECD Guideline 302A (modified SCAS test). EPA agrees with the submitter that these chemicals are not readily biodegradable (test plan, page 16).

RESPONSE: The test was corrected to indicate that the SCAS test was representative of the OECD 302 series inherent tests and not the ready tests. Several additional paragraphs of test were added to the test plan pulling together the information from the primary and supplementary tests. The overall conclusion that these materials are not readily biodegradable but are degradable with time was more clearly stated and supporting evidence was presented. No material changes were made in the robust summaries.

COMMENT 4: *Genetic Toxicity*. EPA reserves judgement on the adequacy of the bacterial mutagenesis assays, pending receipt of robust summaries that identify the test compounds by name.

RESPONSE: Chemical names and CAS Registry numbers have been added for all tested substances. Where available, supplier information, lot numbers and purity information has also been added

COMMENT 5: *Reproductive Toxicity*. EPA reserves judgment on the adequacy of this endpoint for ONCB, pending receipt of a revised robust summary that identifies the organs examined for histopathology, and for PNCB, verification of the compound name.

RESPONSE:

The requested information has been added to the robust summaries

COMMENT 6: Ecological Effects *(fish, invertebrates, and algae*) There were several inconsistencies in the test plan. First, Tables 1, 2, and 3 (pp. 11-13) reported that estimation methods were not available for aquatic toxicity endpoints for ONCB or PNCB or for acute fish toxicity for MNCB. Predicted toxicity values, however, were included in the test plan for each of the three isomers for all three aquatic toxicity endpoints. Second, page 17 reports that ECOSAR predictions were reported for daphnids and algae; however,

predictions for fish were also reported. Finally, Table 6 (p. 17) indicates that a 48-hour algal EC50 was estimated for the MNCB. The estimated EC_{50} value, however, was a 96-hour value.

RESPONSE: Thank you for the careful review and finding these inconsistencies. All of these have been corrected in the revised test plan.

Specific Comments on the Robust Summaries

COMMENT 7: Each summary should clearly identify the test substance by the chemical name. Some robust summaries did not identify the test substance at all and others identified the compound only by its acronym. The submitter needs to revise the summaries, especially for the studies that did not identify the test substance.

RESPONSE: The test substance has been identified in all revised robust summaries. As far as possible, the source and purity of the test substance has also been added.

COMMENT 8: *Genetic Toxicity.* Robust summaries for mutagenesis assays in *Salmonella typhimurium* for the need to specify the test material.

RESPONSE: Chemical names and CAS Registry numbers have been added for all tested substances. Where available, supplier information, lot numbers and purity information has also been added. All were checked for accuracy.

COMMENT 9: *Reproductive Toxicity.* A robust summary for a continuous breeding assay in mice exposed to ONCB by gavage needs to identify the organs examined for histopathology and include separate NOAEL fields for systemic and reproductive toxicity.

The submitter needs to identify the test substance in the summary in the PNCB dossier for a two-generation reproductive toxicity assay in rats, which identified the chemical as ONCB under "Test substance" but as PNCB in the results section and PNCB in the reference list. The submitter needs to include separate NOAELs for systemic and reproductive toxicity.

RESPONSE: The ONCB reproductive robust summary was amended to include separate fields for systemic and reproductive toxicity and the reproductive organs evaluated were listed.

In the PNCB summary, the correct test substance identification has been added as has detailed information about the substance used for the two-gen study including purity, lot number and impurities. A separate conclusion has been added clearly stating the

systemic NOAEL and the reproductive NOAEL. The NOAELs in the definitive field section of the robust summary have been corrected to reflect the results and the conclusions.

The PNCB continuous breeding robust summary was also amended similarly.

COMMENT 10: *Developmental Toxicity*. The summary for the ONCB inappropriately used the ">=" symbol rather than the "=" symbol in the NOAEL fields for doses that were not the highest dose levels.

RESPONSE: This inadvertent symbol use was corrected for the ONCB study.

In addition, the PCNB rat developmental toxicity study has been marked as critical and detailed information has been provided about the test substance. Detailed information about the test substance used in the rabbit developmental toxicity study was also included in the robust summary.

COMMENT 11: ECOSAR predictions were reported for each chloronitrobenzene isomer for all endpoints; however, no details on the inputs used to generate the predictions were reported. Also, the robust summaries indicated that the SAR for esters was used for all of the predictions although none of the sponsored chemicals are esters. From independent model runs, it appears that the submitter correctly used the SAR for neutral organics for toxicity predictions for MNCB. However, it is not clear how the submitter determined the predicted toxicity values for the other two isomers.

RESPONSE:

The ECOSAR modeling was run again using SMILES structures as given in the robust summaries for all three isomers. The original ECOSAR were run with the neutral organics model and not the ester model and this typographical error has been corrected. The original modeling was also run allowing the ECOSAR program to estimate the $K_{o/w}$ that was used in the algorithm. The new calculations were run using the measured $K_{o/w}$ and are thus considered to be better estimates. The ECOSAR calculations are "Critical Studies" for the invertebrate and algal endpoints of MNCB and have been added to the other aquatic robust summaries as supporting data. In all cases the methodology and inputs are clearly shown in the robust summaries.

COMMENT 12: *Fish*. Important details missing from one or more summaries included results based on measured concentrations, values for the actual test concentrations, use

and response of controls, mortality data, 95% confidence intervals, statistical methods, and concentration of the solvent (acetone).

RESPONSE:

Ortho: All available information from this study is provided in the robust summary. The ECOSAR calculation has been shown in detail as supporting data.

Meta: All available information from this study is provided in the robust summary. The ECOSAR calculation has been shown in detail as supporting data.

Para: All available information from this study is provided in the robust summary. Exact purity and CASNO of test substance have been added. The ECOSAR calculation has been shown in detail as supporting data.

COMMENT 13: *Invertebrates*. Important details missing from one or more robust summaries included test substance identity and purity, mortality data, and the concentration of the solvent.

RESPONSE:

Ortho: All available information from this study is provided in the revised robust summary. The ECOSAR calculation has been shown in detail as supporting data.

Meta: The ECOSAR determination has been rerun using the measured Kow value and all parameters have been clearly indicated. The supplemental study lack details but all available information is provided in the robust summary for this published study.

Para: All available information from this study is provided in the revised robust summary. The ECOSAR calculation has been shown in detail as supporting data.

COMMENT 14: *Algae*. Important details missing from one or more robust summaries included test substance purity, type of test (e.g., static, semi-static, or flow-through), pH at the beginning and end of the test, water hardness, specific test concentrations (although ranges were provided), type of regression analysis used to determine the EC_{50} values, and which endpoint (biomass, etc.) was reported.

RESPONSE:

Ortho: All available information from this study is provided in the revised robust summary including the method of regression analysis and type of test. Water hardness is

not necessary as this study used a defined media, which is described in the published reference, prepared from deionized water. PH and endpoint have been addressed. The ECOSAR calculation has been revised to reflect the measured Kow value as an input and has been shown in detail as supporting data.

Meta: The ECOSAR determination has been rerun using the measured Kow value and all parameters have been clearly indicated. The supplemental study lack details but all available information is provided in the robust summary for this supporting published study.

Para: All available information from this study is provided in the revised robust summary including the method of regression analysis and type of test. Water hardness is not necessary as this study used a defined media, which is described in the published reference, prepared from deionized water. The initial pH value has been added. The ECOSAR calculation has been revised to reflect the measured Kow value as an input and has been shown in detail as supporting data.

Environmental Defense Comments

COMMENT 15: The sponsor states that there are no known consumer uses of category members and that emissions are minimal. However, no information was provided on environmental releases (air or water) during the production of different products. It seems plausible that the emissions for some uses of the chloronitrobenzenes might be greater than it is for others. While some of these potential releases would likely come from the facilities of Solutia's customers for these chemicals, we encourage the sponsor to provide environmental release data if available for the different uses of the chloronitrobenzenes.

RESPONSE: The commenter is correct. Data are not available to support the conclusion of minimal release to air and water. This conclusion is not an integral part of the HPV Chemical Challenge, thus it has been removed from the Test Plan.

COMMENT 16: The sponsor states that PCNB is the most toxic of the chloronitrobenzenes; however, this statement is not supported by data presented in the robust summaries. For example, all proposed members induce methemoglobinemia and rat acute toxicity data show that MCNB is the most toxic, followed by OCNB and then PCNB. Moreover, inhalation repeat dose studies indicate no difference in the NOEL for OCNB and PCNB. These studies also show that OCNB induces epithelial hyperplasia of the respiratory tract at low doses, whereas PCNB does not cause this effect even at high doses. Mutagenicity studies indicate that OCNB and PCNB are equipotent. Therefore, the

data indicate that PCNB cannot be considered the most toxic of the chloronitrobenzenes. Rather, in our view, rank ordering done for purposes of read-across needs to reflect the actual toxicity values for a given endpoint for the category members. It should also be assumed that any effect caused by any of the proposed category members will occur for all members.

RESPONSE: This conclusion was drawn in the context of relative potency of these isomers to form methemoglobinemia. See Davydova, SG. 1967. A Comparison of the Properties of Nitrochlorobenzene Isomers for the Determination of Their Permissible Concentrations in Water Bodies. Hyg. and Sanit. 32(8):161-166. for a discussion of the relative toxicity of these three isomers

COMMENT 17: The sponsor states that there is an adequate margin of safety for occupational exposures to the chloronitrobenzenes. This is a risk assessment statement and there is inadequate data presented in the test plan and robust summaries to justify it. We also note that inhalation repeat dose studies on OCNB indicate that hyperplasia of the respiratory epithelium and methemoglobinemia are occurring at a dose of 1.1 ppm -- an exposure level quite close to the TLV. This finding does not indicate an adequate margin of safety.

RESPONSE: This risk assessment statement has been deleted, as risk assessment is not an integral part of the HPV program.

Animal Protection Organizations Comments

No responses necessary

201-14392A

HIGH PRODUCTION VOLUME (HPV)

CHEMICAL CHALLENGE PROGRAM

TEST PLAN

For the

CHLORONITROBENZENE CATEGORY

CAS Number 88-73-3; Benzene, 1-Chloro-2-Nitro-

CAS Number 121-73-3; Benzene, 1-Chloro-3-Nitro-

CAS Number 100-00-5; Benzene, 1-Chloro-4-Nitro-

Prepared by:

Solutia Inc. Registration No.

575 Maryville Centre Drive,
St. Louis, Missouri 63141

EXECUTIVE SUMMARY

Solutia Inc. voluntarily submits the following Category Justification, Screening Information Data (Robust Summaries) and Test Plan for review under the Environmental Protection Agency's High Production Volume (HPV) Chemicals Challenge Program. The category, entitled "Chloronitrobenzenes" consists of three members, Benzene, 1-chloro-2-nitro-, also known as o-Chloronitrobenzene (CAS No. 88-73-3), Benzene, 1-chloro-3-nitro-, also known as m-Chloronitrobenzene (CAS No. 121-73-3), and Benzene, 1-chloro-4-nitro-, also known as p-Chloronitrobenzene (CAS No. 100-00-5). This category is justified on the basis of chemical structure similarity, as well as similarity of basic screening data, as provided in an initial assessment of physico-chemical properties, environmental fate and human and environmental effects.

A substantial amount of data exists to evaluate the potential hazards associated with this Category of chemicals. Use of key studies available from data already developed or derived from recommended estimation models provide adequate support to characterize each Endpoint in the HPV Chemicals Challenge Program without the need for additional testing.

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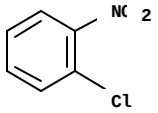
TEST PLAN FOR CHLORONITROBENZENES

I. INTRODUCTION AND IDENTIFICATION OF CATEGORY MEMBERS

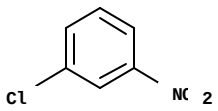
Under EPA's High Production Volume (HPV) Chemicals Challenge Program, Solutia Inc. has committed to voluntarily compile basic screening data on three chemicals of similar structure, namely Benzene, 1-chloro-2-nitro (known as o-chloronitrobenzene or ONCB; CAS no. 88-73-3), Benzene, 1-chloro-3-nitro (known as m-chloronitrobenzene or MNCB; CAS no. 121-73-3) and Benzene, 1-chloro-4-nitro (known as p-chloronitrobenzene or PNCB; CAS no. 100-00-5). Solutia Inc. believes that a category of Chloronitrobenzenes is scientifically justifiable. The data included in this Category involve physicochemical properties, environmental fate, and human and environmental effects of the chemicals in this Category, as defined by the Organization for Economic Cooperation and Development (OECD). Most of the information provided comes from existing data developed on behalf of Solutia Inc., or its predecessor Monsanto Co., much of which has already been submitted to the US EPA under auspices of sections of the Toxic Substances Control Act and is available through TSCATS; additional information can be found in the published scientific literature or from recommended estimation models. This submission fulfills Solutia's obligation to the HPV Challenge Program for these three chemicals.

A. Structure and Nomenclature

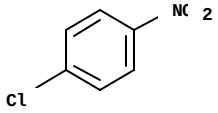
The members of this family of Chloronitrobenzenes, include the following chemicals:



a. Benzene, 1-chloro-2-nitro-
CAS No. 88-73-3
Synonyms: o-Nitrochlorobenzene; o-Chloronitrobenzene; ONCB



b. Benzene, 1-chloro-3-nitro-
CAS No. 121-73-3
Synonyms: m-Nitrochlorobenzene; m-Chloronitrobenzene; MNCB;



c. Benzene, 1-chloro-4-nitro-
CAS No. 100-00-5
Synonyms: p-Nitrochlorobenzene; p-Chloronitrobenzene; PNCB;

B. Manufacturing & Use

Members of the Chloronitrobenzenes Category, p-nitrochlorobenzene (PNCB), o-nitrochlorobenzene (ONCB) and m-nitrochlorobenzene (MNCB), are manufactured by a single US producer, Solutia Inc., at a single manufacturing site in an essentially closed, continuous process. Only a few employees are involved in the manufacturing operations and have minimal potential for skin or airborne exposure, which occurs chiefly during material transfer operations.

All three Chloronitrobenzene isomers, PNCB, ONCB and MNCB are known to produce methemoglobinemia in human and animals (Linch, 1974) and are considered hazardous after dermal contact. Addition of the nitro group in the *para* position relative to the chlorine group on the benzene molecule results in the formation of the most toxic of the three isomers. Potency of response in both humans and animals is equivalent to para> meta>>ortho (Watanabe et al, 1976; Davydova, 1967). To minimize the potential for adverse health effects due to methemoglobinemia resulting from occupational exposure via inhalation or skin absorption, a TLV ® of 0.1 ppm (~0.64 mg/m^3) has been established for PNCB (ACGIH, 2001). While comparative toxicity and occupational experience indicate that MNCB and ONCB produce less toxicity and a lower risk of methemoglobinemia, an internal Solutia Inc. occupational standard of 1 mg/m^3 has also been set for these chemicals. In all cases, specific manufacturing procedures and practices have been established to minimize occupational exposure potential.

PNCB and ONCB are important chemical intermediates that serve as basic building blocks for the manufacture of numerous industrial chemicals. For example, PNCB is utilized via chemical reaction to make industrial chemicals that are ultimately used in the preparation of dyes and pigments, pesticides, and animal feed ingredients. ONCB is converted in similar fashion to dyes and pigments, polymer additives, veterinary pharmaceuticals and water-treatment chemicals. MNCB has limited use as a chemical intermediate.

Chloronitrobenzenes are sold to a limited number of customers at a few processing sites for the express purpose of full chemical conversion into other industrial chemicals. There are no known or suspected consumer exposures to these chemicals resulting from TSCA-related activities, as they are fully consumed as chemical intermediates.

II. CATEGORY JUSTIFICATION

For purposes of the HPV Challenge Program, EPA has provided guidance as to the definition and justifications to be used in selection of a chemical Category (US EPA, 1999c). The definition states that a chemical Category should be "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". Solutia Inc. has opted to form the Chloronitrobenzene Category with this guidance in mind.

Common Structure

The three chemicals selected for inclusion in this category are isomeric forms of the same base chemical, nitrobenzene. Hence, they are of common structure.

Common Functional Groups

Each of these nitrobenzene compounds are aromatic hydrocarbons for which one benzene ring hydrogen has been replaced by a nitro (NO2) radical and one benzene ring hydrogen further replaced with a chloro (Cl) group; the position (either *ortho* to, *meta* to, or *para* to the chloro grouping) of the ring placement of the nitro grouping is the only structural difference between these three isomers. For the most part, these compounds are similar in chemical properties, as well as in their pharmacological or toxicological effects. As such these effects are modified to a greater or lesser degree by the location of the substituent radicals (Beard and Noe, 1982; Davydova, 1967; Watanabe et al, 1976).

Similar or even Identical Properties or Hazards

Physicochemical properties of these three isomeric forms of the same chemical are quite similar. Their physical form is crystalline and their molecular weights and specific gravity are identical. Other parameters are similar, but not identical. A summary of available physicochemical data can be found in Table 4.

Environmental Fate data are summarized in Table 5. A large body of published information exists in this data category. Whether measured or estimated, there appears close agreement in each of the HPV Endpoints recorded for each of the chemicals in this category.

Comparative aquatic toxicity of the members of this Category can be found in Table 6. As shown, a similar degree of toxicity has been observed across the multiple test species included in this dataset.

Tables 7 - 10 summarize the comparative mammalian toxicity of these chemicals. It is well recognized that all three of these chemicals possess a similar mode of action. Their toxicity is characterized by a common and outstanding property, i.e., their ability to form methemoglobin (Beard and Noe, 1982) in both humans and animals. Comparative investigations have established the order of potency to be: para isomer > meta isomer >> ortho isomer (Watanabe et al, 1976; Davydova, 1967). However, there are marked species differences in susceptibility to methemoglobinemia with humans being decidedly more affected than rodent species. Thus, results of acute toxicity studies in rodents are not considered fully representative of the high acute toxicity to humans that can be elicited by these chemicals. On the basis of past human experience, where dermal contact or inhalation exposures resulted in incidences of methemoglobinemia, unusually diligent care has been taken to insure proper handling of both chemicals (each treated equally) during manufacture, shipment, disposal and use.

Thus, similarities in the chemical structure, biological mode of action and the extensive comparative data sets presented support use of a Category approach for these chemicals.

III. TEST PLAN RATIONALE

The information obtained and included to support this Test Plan have come from either 1) internal studies conducted by/or for Solutia Inc. (or its predecessor Monsanto Co.), 2) have been extracted from the scientific literature either as primary references or as found in well-accepted, peer-reviewed reference books, or 3) were estimated using environmental models accepted by the US EPA (1999b) for such purposes. This initial assessment includes information on physicochemical properties, environmental fate, and human and environmental effects associated with each member of this Category. The data used to support this program include those endpoints identified by the US EPA (1998); key studies have been identified for each data Endpoint and summarized in Robust Summary form and included in Section VII of this dossier.

All studies were reviewed and assessed for reliability according to standards specified by Klimisch *et al* (1997), as recommended by the US EPA (1999a). The following criteria were used for codification:

1. Reliable without Restriction - Includes studies which comply with US EPA and/or OECD-accepted testing guidelines, which were conducted using Good Laboratory Practices (GLPs) and for which test parameters are complete and well documented,

2. Reliable with Restriction – Includes studies which were conducted according to national/international testing guidance and are well documented. May include studies conducted prior to establishment of testing standards or GLPs but meet the test parameters and data documentation of subsequent guidance; also includes studies with test parameters which are well documented and scientifically valid but vary slightly from current testing guidance. Also included were physical-chemical property data obtained from reference handbooks as well as environmental endpoint values obtained from an accepted method of estimation (i.e. EPIWIN).

3. Not Reliable – Includes studies in which there are interferences in either the study design or results that provide scientific uncertainty or where documentation is insufficient.

4. Not Assignable – This designation is used in this dossier for studies which appear scientifically valid but for which insufficient information is available to adequately judge robustness.

Those studies receiving a Klimisch rating of 1 or 2 are considered adequate to support data assessment needs in this Dossier. Those key studies selected for inclusion are considered typical of the Endpoint responses observed in other studies of a similar nature and design, which were identified during our search of the literature; additional references can be found in the current ECB IUCLID dossiers for o-Chloronitrobenzene (2000), m-Chloronitrobenzene (2000) and p-Chloronitrobenzene (2000), as referenced below.

IV. TEST PLAN SUMMARIES AND CONCLUSIONS

The referenced available data for each Category member have been placed in an Endpoint-specific matrix and summarized individually in Table 1 (ONCB), Table 2 (MNCB) and Table 3 (PNCB). Substantial data exists for each chemical to evaluate its potential hazards in this screening level assessment. Where an HPV Endpoint has been untested, the need for testing has been assessed (1) with the understanding that these chemicals behave in a similar and/or predictable manner, and (2) by interpolation (i.e. Read-Across technique) between data from other key studies already available. Thus, we have used preexisting data, where possible, to support our assessment of potential hazards of the chemicals in this Category and avoid the unnecessary testing of additional laboratory animals.

Conclusion: All HPV Endpoints have been satisfied for the three Chloronitrobenzene isomers with data from studies that were either well documented, used OECD guideline methods and conducted in accord with GLPs, or were estimated from acceptable estimation modeling programs. Use of the "Read Across" technique was employed sparingly to support a limited number of endpoints. Hence, no further testing for any of the HPV endpoints is deemed necessary (Tables 1, 2 and 3).

Physical-chemical property values - Melting Point and Boiling Point values for all three Chloronitrobenzenes were obtained from reputable references and cited as an Accepted or Peer Reviewed value in their respective Hazardous Substances Data Banks (2002). Measured values were found for Vapor Pressures and Partition Coefficients from reputable studies, and which were also cited in accepted peer reviewed documents. Experimental values for the water solubility of each isomer have been published and are supported by an accepted estimation method . These values were given a classification of "2-Reliable with restrictions".

Environmental Fate values describing Transport (Fugacity) for ONCB, MNCB and PNCB were obtained using a computer estimation –modeling program (EPIWIN, 2002) recommended by EPA and classified as "2-Reliable with restrictions".

Photodegradation (direct) and Biodegradation data for each of the three Chloronitrobenzene isomers were characterized in well-documented studies, the latter conducted according to ASTM/EPA guidelines that since have been codified and are similar to OECD test #301 guidance. These studies thus are classified as "2-Reliable with restrictions" Indirect photodegradation was estimated with EPIWIN and separate robust summaries for indirect photodegradation are included. . No specific data were found for water stability of the chloronitrobenzenes. The hydrolysis of chloronitrobenzenes to chlorophenols (hydrolysis of the nitro group) or to nitrophenols (hydrolysis if the chloro group) are both thermodynamically feasible, as the enthalpy of reaction calculated from bond energies indicates hydrolysis to be a thermodynamically favored process (see robust summaries). The free energy of the transition state for this hydrolysis; however, is so high that the reactions are generally not feasible (March's Advanced Organic Chemistry, fifth ed 2001 page 433). Special aromatic compounds, such as those with multiple electron withdrawing groups ortho and para to the halogen are potentially activated; however, even picryl chloride (with three nitro groups ortho or para to the chlorine) is stable in water at room temperature as it is transported with about 10% water to limit explosion potential. Thus, ortho and para CNB are anticipated to be hydrolysable under extreme conditions but are considered to have a hydrolytic half-life greater than one year under environmental conditions and the meta isomer is expected to be even more stable to hydrolysis. As chemical principles are considered reliable methodology, the reliability score is assigned as "2".

Ecotoxicity – Acute Fish, Invertebrate and Plant Toxicity Endpoints for PNCB and ONCB have been fulfilled with studies, most of which were conducted according to US EPA test guidance consistent with OECD test guidelines. All studies were well documented and were designated "2-Reliable with restrictions". An Acute Fish Toxicity study, also designated as "2-Reliable with restrictions", has been included for MNCB. The Acute Invertebrate and Plant Toxicity Endpoints for MNCB are fulfilled using the 'Read Across" method of data evaluation, as no fully reliable studies were found in these two areas. Utility of this methodology is strengthened by comparative use of estimation modeling data as well as literature information deemed limited ("4-Not Assignable") in documentation, but useful for supportive purposes.

Mammalian Toxicity Endpoints, including Acute Toxicity, Repeated Dose Toxicity, Ames Mutagenicity, Chromosomal Aberration Testing and Reproductive Toxicity for both PNCB and ONCB have been fulfilled by way of tests that either conformed directly to OECD test guidance or followed test designs similar to OECD guidance. Thus, they have been designated either "1-Reliable without restriction" or "2-Reliable with restrictions".

An Acute Toxicity study, an Ames test and a Cytogenetics study have been conducted with MNCB and fulfill these Endpoint requirements for this isomer; each of these studies has been designated as either "1-Reliable without restriction" or "2-Reliable with restrictions". No Repeated Dose Toxicity (of sufficient reliability) or

Reproductive Toxicity studies have been identified for MNCB. Thus, these Endpoints have been filled using the "Read Across" technique for data assessment, since both the ortho and para isomers have been extensively evaluated for these Endpoints.

Based on the conclusions as outlined above on HPV Endpoint assessment, following is a tabular depiction of data availability and testing recommendations for ortho-Chloronitrobenzene (ONCB) (Table 1), meta-Chloronitrobenzene (MNCB) (Table 2) and para-Chloronitrobenzene (PNCB) (Table 3).

Table 1. Test Plan Matrix for ortho-Chloronitrobenzene (ONCB)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept-Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	N	Y	N
Boiling Point	Y	N	N	R	N	Y	N
Vapor Pressure	Y	N	N	R	N	Y	N
Partition Coefficient	Y	N	N	R	N	Y	N
Water Solubility	Y	N	N	R	N	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	Y	N/Y	Y	N
Stability in Water	Y	N	N	N	Y	Y	N
Biodegradation	Y	N	N	Y	N	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	N	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	N	N	Y	N	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	N	Y	N	Y	N
Acute Toxicity to Aquatic Plants	Y	N	N	Y	N	Y	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	N	N	Y	N	Y	N
Repeated Dose Toxicity	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Mutation (Ames)	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	N	Y	N	N	Y	N
Reproductive Toxicity	Y	N	Y	N	N	Y	N
Developmental Toxicity	Y	Y	Y	Y	N	Y	N

Y = Yes; N = No; R = Reputable Reference; - = Not applicable

Table 2. Test Plan Matrix for meta-Chloronitrobenzene (MNCB)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept-Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	N	Y	N
Boiling Point	Y	N	N	R	N	Y	N
Vapor Pressure	Y	N	N	R	N	Y	N
Partition Coefficient	Y	Y	Y	R	N	Y	N
Water Solubility	Y	N	N	R	N	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	Y	N/Y	Y	N
Stability in Water	Y	N	N	N	Y	Y	N
Biodegradation	Y	N	N	Y	N	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	N	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	N	N	Y	N	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	N	Y	Y	C	N
Acute Toxicity to Aquatic Plants	Y	N	N	Y	Y	C	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	Y	Y	Y	N	Y	N
Repeated Dose Toxicity	Y	N	N	Y	N	C	N
Genetic Toxicity – Mutation (Ames)	Y	N	N	Y	N	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	N	Y	N	N	Y	N
Reproductive Toxicity	N	-	-	-	-	C	N
Developmental Toxicity	N	-	-	-	-	C	N

Y = Yes; N = No; R = Reputable Reference; ; - = Not applicable

C = Read-across from available data on ONCB & PNCB

Table 3. Test Plan Matrix for para-Chloronitrobenzene (PNCB)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept-Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	N	Y	N
Boiling Point	Y	N	N	R	N	Y	N
Vapor Pressure	Y	N	N	R	N	Y	N
Partition Coefficient	Y	N	N	R	N	Y	N
Water Solubility	Y	N	N	R	N	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	Y	N/Y	Y	N
Stability in Water	Y	Y	Y	-	Y	Y	N
Biodegradation	Y	N	N	Y	N	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	N	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	N	Y	Y	N	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	Y	Y	N	Y	N
Acute Toxicity to Aquatic Plants	Y	N	N	Y	N	Y	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	N	N	Y	N	Y	N
Repeated Dose Toxicity	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Mutation (Ames)	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	Y	Y	Y	N	Y	N
Reproductive Toxicity	Y	Y	Y	Y	N	Y	N
Developmental Toxicity	Y	Y	Y	Y	N	Y	N

Y = Yes; N = No; R = Reputable Reference; - = Not applicable

V. Data Set Summaries and Evaluations

The key studies used in this assessment to fulfill the HPV requirements for ONCB, MNCB and PNCB have been placed in an Endpoint-specific matrix, and further discussed below. As a number of studies supporting many of these Endpoints exist for each Chloronitrobenzene, key studies were selected based on their representative presentation of data characterization as well as their reliability. Robust Summaries for each study referenced can be found in Section VII of this dossier.

A. Chemical/Physical Properties

A large number of studies are available summarizing the **Physical-Chemical** properties associated with these Chloronitrobenzenes. They can be found in ECB IUCLID Dossiers for o-Chloronitrobenzene (2000), m-Chloronitrobenzene (2000) and p-Chloronitrobenzene (2000). Table 4 contains those values that are considered to best depict the consensus of results found in most key sources used to define the characteristics of each of these Chloronitrobenzenes. They have been obtained from reputable reference books or measured values and cited in peer-reviewed data sources; thus, they are considered "2-Reliable with restrictions". A Robust Summary has been prepared for each of the references included in Table 4.

In summary, ONCB, MNCB, and PNCB are solid entities at room temperature and possess low vapor pressures. They have a moderate partition coefficient and are moderately soluble in water.

Conclusion: Sufficient data exists to fully characterize the Physicochemical properties of each of these Chloronitrobenzenes. All HPV data requirements for this Endpoint have been met and no further data collection is planned.

Table 4. Selected Physical Properties of Chloronitrobenzenes

Chemical	Boiling Pt. (^{o}C.)	Melting Pt. (^{o} C.)	Vapor Pressure (hPa @ 25 ^{o}C)	Water Solubility (mg/L)	Partition Coeffient (Log Kow)
o-Chloronitrobenzene CAS No. 88-73-3	245.7	32.5	0.0575 @ 20°C	198 @ 25°C	2.24
m-Chloronitrobenzene CAS No. 121-73-3	236	46	0.129	273 @ 20°C	2.49
p-Chloronitrobenzene CAS No. 100-00-5	242	83.4	0.1253	189.4 @ 25°C	2.39

B. Environmental Fate and Biodegradation

Semi-Continuous Activated Sludge (SCAS) Biodegradability studies have been conducted to assess the biodegradation potential of ONCB and PNCB; they have been summarized in the Robust Summary section of this Dossier and cited in Table 5 below. While each study was conducted prior to inception of standardized international guidelines for **Biodegradability** testing and GLPs, they followed similar standards for conduct subsequently codified into OECD guideline 302 (Inherent Biodegradation) and GLP documentation. Thus, they are each considered "2-Reliable with restrictions". An anaerobic bacterial assay with MNCB was selected to fulfill this HPV data requirement as it was well documented and thus also considered "2-Reliable with restrictions". Supplemental studies summarized in Section VII for each compound confirm the conclusion that Chloronitrobenzenes undergo slow biodegradation in non-adapted soil.

Literature data for ONCB are somewhat conflicting. A report by Zoeteman (1980) indicated that ONCB has an estimated river water half-life in of 3.2 days based on monitoring data from the Rhine River. Another river study, however, indicates that this compound can travel long distances in surface waters (900 miles in the Mississippi River) at concentrations that are explained by simple dilution (Howard, et al. 1976). The SCAS test result of 11 to 48% removal in 24 hours is more consistent with the Zoeteman result. ONCB does not appear to be readily biodegradable but is probably degradable in the environment with time.

The literature indicates that MNCB is resistant to aerobic biodegradation. In aerobic tests using both adapted and unadapted bacteria, Canton et al (1985) found that the half-life was much greater than 4-weeks using either inoculum. The anaerobic test presented in the robust summary shows only that primary degradation takes place under anaerobic conditions. This is not surprising, as the nitro group is expected to undergo facile reduction. The other tests presented in the Robust Summaries are supportive of poor biodegradation. Overall, it can be concluded that MNCB is not readily biodegradable.

PNCB half-life in the Rhine River was also investigated by Zoeteman (1980) who reported a half-life between 0.3 and 3.0 days. The SCAS test result of 34 to 66% removal in 24 hours is consistent with the Zoeteman result. The supplemental studies are also supportive of PNCB being somewhat more easily biodegraded than the ortho isomer. PNCB does not appear to be readily biodegradable but is likely degradable in the environment with time.

A single, comparative study of the photochemical reactions associated with each of the three Chloronitrobenzenes has been summarized in the Robust Summary section of this dossier. It has been classified as "2-Reliable with restrictions", as it provides useful information, appears well conducted, but did not conform to codified OECD guidelines. Comparative values have been included in Table 5. AOPWIN modeling for this **Photodegradation** Endpoint has also been included to estimate atmospheric indirect

photodegredation. We have incorporated the use of an estimation model (EPIWIN, 2002) for determination of Transport Between Environmental Compartments (**Fugacity),** for all three Chloronitrobenzenes**.** A Fugacity Level III model was used in each case, and employed measured values, where possible, as recommended by the US EPA. Thus, the estimations derived from each of these models have been classified as "2-Reliable with restrictions". These estimates have also been included in Table 5 and are cited in the Robust Summary section of this Dossier; data entries used in the Level III fugacity model have been included in the Robust Summaries for validation of output.

No values have been identified to define the **Stability in Water** (hydrolysis) of any of these Chloronitrobenzenes. Further no such values could be calculated using EPIWIN (2002) as each chemical has only aromatic nitro and aromatic chloro functional groups, both of which are listed in Lyman et al. (1990) as Generally Resistant to Hydrolysis. Thermodynamic calculations, however, have been conducted and included in robust summary form to support the hydrolytic stability of these materials in water. Thus, "[t]esting for Stability in Water is not needed for substances generally recognized to have molecular structures or possess only functional groups that are generally known to be resistant to hydrolysis" (OECD, 2002).

Conclusion: Sufficient information exists to characterize the Environmental Fate and Biodegradation of each of these Chloronitrobenzenes. Where experimental data do not exist, use of an estimation model (EPIWIN) recommended by EPA provided necessary information or the rationale lack of need for testing has already been recognized or calculated based on thermodynamics. Thus, all HPV data requirements for this Endpoint are met and no further data collection is planned.

Table 5. Comparison of Environmental Fate Endpoints for Category Members

Chemical	Biodegradation Rate	Stability in Water	Direct Photodegradation (% Disappeared-5 Hr Irradiation)	Fugacity (%)
o-Chloronitrobenzene CAS No. 88-73-3	11-48 % Primary Degrad.(SCAS)	n.d.	66	Air- 6.5 Water- 33.5 Soil- 59.8 Sediment-0.16
m-Chloronitrobenzene CAS No. 121-73-3	50% (anaerobic sediment)	n.d.	89	Air- 8.0 Water- 28.8 Soil- 63.0 Sediment-0.19
p-Chloronitrobenzene CAS No. 100-00-5	31-66% Primary Degrad. (SCAS)	n.d.	96	Air- 9.5 Water- 28.5 Soil- 61.8 Sediment- 0.17

nd. = no data available

To summarize the Environmental fate of these Chloronitrobenzenes, based on Fugacity modeling the members of this Category are expected to be found primarily in the soil and water as main environmental target compartments. None of these chemicals is readily hydrolysable in the environment. They can be abiotically reduced in the presence of natural electron transport mediators and under reducing conditions, but are not Readily Biodegradable. Under conditions of domestic waste treatment, considerable biodegradation is apparent. Estimated Koc values suggest the Chloronitrobenzenes possess moderate mobility in soils (EPIWIN, 2002); slow volatilization is expected to occur, based on their vapor pressures. These chemicals are expected to exist primarily in the vapor phase in the atmosphere where they will degrade slowly by reaction with photochemically producing hydroxyl radicals.

C. Aquatic Toxicity

Several references to acute fish, invertebrate and algal toxicity can be found in the ECB IUCLID documents for ONCB (2000), MNCB (2000) and PNCB (2000). Data presented in Table 6, and summarized in the Robust Summary section VII, depict the level of toxicity generally observed for these Endpoints within the overall dataset. All of the studies selected to fulfill the Acute Fish, Acute Invertebrate and Acute Plant Toxicity Endpoints for ONCB and PNCB were either conducted according to US EPA test guidance (ASTM/EPA) consistent with international guidance or published in a peer-reviewed journal possessing sufficient documentation. Thus, they are considered "2-Reliable with restrictions". Similarly, a well-documented Acute Fish toxicity study with MNCB, which followed US EPA/ASTM guidance, is also considered "2-Reliable with restrictions". Two literature articles were found summarizing acute toxicity effects of MNCB in Daphnia and algae. Both purportedly were conducted following OECD or Dutch National testing guidance. Additionally, both articles provided a comparative assessment of all three Chloronitrobenzene isomers considered in this Category. However, neither article provides sufficient detail nor individual data documentation to be assigned a reliability code other than "4- Not assignable" for HPV purposes. A Robust Summary has been completed for each study and included in the Robust Summary Section of all three isomers in Section VII of this Dossier.

Additionally, we have conducted estimation modeling for aquatic toxicity endpoints on all three isomers. Where acceptable measured data were available, these data were used as the critical study, with ECOSAR estimates as supporting information. Where information on measured data were not sufficient (MNCB 48hr. Daphnid EC50 and 96hr. Algal EC50), the ECOSAR results were considered the critical study.

In summary, the empirical data derived from testing and the estimations derived from modeling, support a similar degree of comparative acute aquatic toxicity of all three Chloronitrobenzene isomers to these three aquatic species. Thus, it is reasonable and justifiable to use the "Read Across" technique for fulfilling both the Acute Invertebrate

and Acute Aquatic Plant Toxicity Endpoints for MNCB from empirically derived data available for both ONCB and PNCB.

Conclusion: Sufficient data exists to fully characterize the Acute Aquatic Toxicity properties of each of these Chloronitrobenzenes. All HPV data requirements for this Endpoint have been met with empirical data or through limited and scientifically justified "Read Across" methods such that no further data collection is required for these materials.

Table 6. Comparison of Aquatic toxicity parameters for category members

Chemical	Fish LC 50 (mg/L) (96-hr)	Invertebrate (Daphnia) LC50 (mg/L) (48-hr)	Algae EC50 (mg/L) (48-hr)
o-Chloronitrobenzene CAS No. 88-73-3	30.03 (14-day) (Guppy)	41.0	34.0 (biomass)
m-Chloronitrobenzene CAS No. 121-73-3	18.8 (F. minnow)	44.8 (estim.)	28.8 (96 hour estim.)
p-Chloronitrobenzene CAS No. 100-00-5	6.0 (R. trout)	10.0	8.0 (biomass)

D. Mammalian Toxicity

1.0 Acute Toxicity

Key acute toxicity studies by the oral exposure route were chosen from a number of other acute reports; these results represent acute toxicity values identified from reliable sources. It should be noted that acute toxicity studies with most laboratory animals are not considered sufficiently predictive of the acute hazards of these nitroanilines to humans, due to the resistance observed in lab animals to development of methemoglobinemia. All studies included in Table 7 were conducted specifically or in general agreement with OECD acute toxicity testing guidance and are considered either "1-Reliable without restriction" or "2-Reliable with restrictions". Other acute toxicity study results are cited in the ECB IUCLID dossiers for ONCB (2000), MNCB (2000) and PNCB (2000).

Table 7. Acute Mammalian Toxicity for Category members

Chemical	Rat Oral LD50 (mg/kg)
o-Chloronitrobenzene CAS NO. 88-73-3	560
m-Chloronitrobenzene CAS No. 121-73-3	400
p-Chloronitrobenzene CAS No. 100-00-5	530

Conclusion: Sufficient data from well-documented studies (Acute Oral Toxicity) exist to meet the Acute Toxicity data set requirements for all members of this Category. Hence, no further acute toxicity testing is planned.

2.0 Repeated Dose Toxicity

PNCB and ONCB have been extensively evaluated in Repeated Dosing studies of various durations and by different exposure routes (ECB IUCLID - PNCB, 2000; ECB IUCLID – ONCB, 2000). Studies conducted in rats for 13 weeks by the inhalation exposure route with ONCB and PNCB, each consistent with OECD Test Guideline 413, have been selected to fulfill the requirements for this HPV Endpoint. Each of those studies is summarized in Table 8, is considered "1-Reliable without restriction" and has been included in the Robust Summary section of this dossier. Additional Repeated Dose rat inhalation studies of a shorter duration (4-weeks), have been included as Supplemental information in Table 8 and summarized in the Robust Summary section of this dossier, as they are useful for comparative purposes. Additionally, it should be noted that other Repeated Oral Dose studies with PNCB are available and have previously been submitted to EPA and are cited in the ECB IUCLID – PNCB (2000). These studies include: a chronic/carcinogenic oral rat study (Nair et al, 1989), a 13-week oral toxicity study in rats (Solutia, 1979).

No adequately reported Repeated Dose studies were found for MNCB after an extensive literature search as well as review of its ECB IUCLID (2000) document. However, the summary of a series of studies comparing MNCB repeated dose toxicity with that of PNCB and ONCB was found (Davydova, 1967). It has been included in this discussion as it provides some useful Supplemental information. Due to its inclusion as only summary data, it has been assigned a Reliability classification of "4-Not Assignable". While included in the Robust Summary section of this dossier, it has not been included in Table 8.

Conclusion: The Repeated Dose HPV Endpoint for both PNCB and ONCB are complete with selection of a 13-week inhalation study in rats for each chemical, as each meets OECD Test Guideline 413; thus, no further testing is needed.

It is scientifically justifiable to consider completion of the Repeated Dose HPV Endpoint for MNCB through use of the "Read Across" technique for data assessment, based on 1) similarity of structure, i.e. it is one of three nitrobenzene isomers considered in this dossier, 2) substantive and fully adequate testing for this Endpoint already exists for the other two isomeric forms, PNCB and ONCB, 3) there is a known, identical mode of action associated with all three isomers (methemoglobinemia) and 4) a consistent pattern of repeated dose toxicity has been established among the three isomers. Clinical observations, serum chemistry changes, organ weight differences and histopathological findings associated with PNCB and ONCB were related to methemoglobin formation and

compensatory processes that occurred as a result. The single Supplemental study found in the literature with MNCB characterized its repeated dose toxicity as fully comparable with that seen with PNCB and ONCB. However, the degree of potency of MNCB was characterized as closer to the more toxic isomer, PNCB, rather than ONCB, the lesser toxic isomer.

Conclusion: "Read Across" methodology, based on the use of reliable data from PNCB and ONCB, is scientifically justified to adequately characterize the Repeated Dose hazards associated with MNCB. Thus, the requirements for the Repeated Dose HPV Endpoint for MNCB are complete and no further, unnecessary animal testing is warranted.

Table 8. Repeated Dose Toxicity Studies with Category Members

Chemical	Study Type	Dosages	Histopathology	Hematology/Clinical Findings
o-Chloronitro-benzene CAS NO. 88-73-3	13-Week Rat Inhalation 10M/10F/group F344 rats	18 ppm	Respir. Epithel.-hyperplasia Liver-basophilia Spleen-congestion Kidney-hemosiderosis Kidney, Liver,Spleen Wt	MET, RETIC, SDH, LB,ALT, AP, B acids HCT, HGB, RBC, PLAT
		9 ppm	Respir. Epithel.-hyperplasia Liver-basophilia Kidney-hemosiderosis Kidney, Liver Wt	MET, RETIC, SDH, LB,ALT, AP, B acids HCT, HGB, RBC, PLAT, MCHC/MCH(F)
		4.5 ppm	Respir. Epithel.-hyperplasia Liver-basophilia Kidney-hemosiderosis Spleen Wt	MET, SDH, ALB,ALT, B acids HCT, HGB, RBC
		2.3 ppm	Respir. Epithel.-hyperplasia Liver Wt	MET, SDH, ALB,ALT, B acids; HCT
		1.1 ppm	Respir. Epithel.-hyperplasia	MET
o-Chloronitro-benzene CAS NO. 88-73-3	4-Week Rat Inhalation 15M/15F/group S-D Rats	60 mg/m3 (~9.3 ppm)	Spleen-Extramed. Hematopoiesis & hemosiderosis Liver, Kidney, & Spleen Wt	MET, RET HCT, HGB, RBC
		30	Spleen-Extramed.	MET

		mg/m3 (~4.6 ppm) 10 mg/m3 (~1.5 ppm)	Hematopoiesis & hemosiderosis Liver, Kidney, & Spleen Wt Liver Wt (M)	HCT (F), HGB (F), RBC (F) -
m-Chloronitro-benzene CAS No.121-73-3		No Data		
p-Chloronitro-benzene (PNCB) CAS No.100-00-5	13-Week Rat Inhalation 10M/10F/group F344 rats	24 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis/fibrosis-hyperplasia Testes - atrophy Liver, Spleen, Heart, Thymus, Testes weights	MET, RET, MCH, n-RBC, SDH, B acids HCT, RBC, HGB, AP, GLOB, ALT, TPROT
		12 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis/fibrosis-hyperplasia Liver, Spleen, Heart weights	MET, RET, n-RBC, SDH, B acids HCT, RBC, HGB, AP, GLOB, ALT, TPROT
		6 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis/fibrosis-hyperplasia Liver, Spleen weights	MET, RET, n-RBC, SDH (F), B acids (M) HCT, RBC, AP, GLOB, ALT, TPROT
		3 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis Liver, Spleen weights	MET, RET, n-RBC, B acids (M) HCT, HGB, RBC, ALT (M)
		1.5 ppm	Renal-hyaline droplets (M only) Spleen -hemosiderosis	MET, RET, n-RBC HCT, HGB, RBC,

				ALT (M)
p-Chloronitro-benzene (PNCB) CAS No.100-00-5	4-Week Rat Inhalation 15M & 15F/group S-D rats	45 mg/m3 (~ 7 ppm)	Spleen-congestion & hemosiderosis & Extramedullary hematopoiesis Liver & Spleen weight	MET HCT, HGB, RBC
		15 mg/m3 (~ 2.3 ppm)	Spleen – hemosiderosis Liver weight (F)	MET HCT, HGB, RBC
		5 mg/m3 (~ 0.8 ppm)	Spleen - hemosiderosis	HCT, HGB, RBC

3.0 Mutagenicity and Chromosomal Aberrations

Ames Test

For each of the three Chloronitrobenzene isomers, a key point mutation study has been selected to fulfill this HPV Endpoint. Both the ONCB and PNCB studies were conducted according to GLPs and conformed to OECD Test Guideline 471 and thus are considered "1-Reliable without restriction". The study with MNCB was well documented but conducted prior to OECD Test Guideline codification and thus is considered "2-Reliable with restrictions". Each study has been cited in Table 9 andsummarized in the Robust Study section of this Dossier. Additional Ames test assays are reported in the ECB IUCLID for ONCB (2000), MNCB (2000), and PNCB (2000).

Weak positive responses were seen in Salmonella with ONCB and PNCB but not MNCB. Both ONCB and PNCB have been consistently inactive (negative) in *in vitro* assays using mammalian cell lines, including the CHO/HGPRT assay (Solutia 1982a, 1983a), the UDS Rat Hepatocyte Culture assay (Solutia 1983b, 1984) and a rat hepatocyte DNA repair assay with PNCB (Solutia, 1982b). PNCB was positive only with metabolic activation in

the Mouse Lymphoma assay (Solutia, 1981). Neither PNCB nor ONCB induced sex-linked recessive lethal germ cell mutations in an *in vivo*, secondary tier mutation assay (NTP, 1993).

Conclusion: The Ames Test Category Endpoint for each of the Chloronitrobenzenes has been met and no further testing should be considered for the gene point mutation endpoint for this chemical.

Table 9. Genetic Toxicity of Category Members

Chemical	Ames Test- TA98, 100, 1535, 1537 +/- activation	Cytogenetics In Vitro (CHO Cells)	Cytogenetics In Vivo
o-Chloronitro-benzene CAS NO. 88-73-3	Positive – TA100 w S-9 Negative – TA100 w/o S-9 Negative -. w & w/o S-9. TA98, TA1535, TA1537	Weak Positive- w S-9 Negative – w/o S-9	 n.d.
m-Chloronitro-benzene CAS No. 121-73-3	Negative – TA100, TA98, TA1535, TA1537, TA1538 w and w/o S-9	Negative - w & w/o S-9	n.d.
p-Chloronitro-benzene CAS No, 100-00-5	Positive – TA1535 w/o S-9 Ambiguous- TA1535 w S-9 Negative – TA98, TA1537, TA100 w and w/o S-9	Weak Positive – w & w/o S-9	Negative

n.d. = no data

Chromosomal Aberrations -

Three *in vitro* CHO cell chromosomal aberration studies sponsored by the US NTP program, each with a different Chloronitrobenzene isomer, have been conducted following a study design similar to, but not identical with, OECD Test guideline 473. Each study was well documented and followed GLPs and thus is considered to be "2-Reliable with restrictions". These studies have been used to fulfill this HPV Endpoint for ONCB and MNCB. However, while the CHO cell study could be used to support this Endpoint for PNCB, a secondary tier, *in vivo* Chromosomal Aberration Test (classified "2-Reliable with restrictions") has been chosen as the key HPV study for this chemical.

Conclusion: On the basis of reliable in vitro (ONCB and MNCB) and in vivo (PNCB) Chromosomal Aberration Assays available for each of these Chloronitrobenzenes, no additional testing is needed to fulfill this HPV Endpoint.

4.0 Reproductive and Developmental Toxicity

PNCB, the most toxic chemical in this Chloronitrobenzene group, has undergone testing for developmental toxicity in two species (rat and rabbit) and has been evaluated both in a rat Two-Generation Reproduction study and a mouse Continuous Breeding study. Each of these studies have been assessed as "1-Valid without restriction" as they fully met OECD testing guidelines (or standardized methodology as in the case of the Continuous Breeding study) and GLP guidance. The Two Generation Rat Reproduction study has been selected as the key study to fulfill the reproductive toxicity endpoint for PNCB as its design is considered more conventional than the Continuous Breeding study. The rat developmental toxicity study has been marked as key for the developmental toxicity endpoint and the rabbit study is included as Supplemental information. Each of these adequately conducted studies has been summarized in Table 10 and Robust Summaries have been developed.

ONCB has been evaluated in a comparative (to PNCB) rat developmental toxicity study filling this HPV endpoint. This study has also been evaluated as being "1-Valid without restriction" and has been summarized in Table 10. Additionally, relative to the reproductive toxicity endpoint, it has been tested in a mouse Continuous Breeding study, as has PNCB. As the Continuous Breeding study was conducted in accord with standardized testing methodology for this reproduction study and under GLPs, it has been classified as "1-Reliable without restriction" and fulfills the Reproductive Toxicity HPV Endpoint for ONCB. Robust Summaries for each study can be found in Section VII of this Dossier.

To summarize the available information on these two Chloronitrobenzene isomers, ONCB was judged "not to be a reproductive toxicant, even in the presence of systemic toxicity in Swiss CD-1 mice" (NTP, 1993). PNCB produced no effects on reproductive toxicity parameters through 2 generations in rats up to a level (5 mg/kg/d) known to produce significant systemic toxicity (Nair et al, 1989). Significant and progressive deficits in infertility in the FO generation and reduced weight gains in F1 and F2 pups were seen in mice during the Continuous Breeding study and may have been related to methemoglobin-related hypoxia associated with cyanosis observed at PNCB test levels. Developmental toxicity was seen only at the highest dose tested in rats with PNCB, and thus was judged to not to have a primary effect on fetal development. ONCB produced no developmental toxicity when evaluated in rats even at maternally toxic levels.

No Reproductive Toxicity or Developmental Toxicity studies have been identified with MNCB. However, we believe sufficient data exists in this Category to obviate the need for further evaluation of MNCB, based on the similarity of mammalian toxicity of this group of Chloronitrobenzene isomers and through use of the

corresponding reproductive toxicity data available on both PNCB and ONCB. A "Read Across" approach, using the PNCB and ONCB reproductive studies in rats and mice, has been used to fulfill the Reproductive Toxicity HPV Endpoint for MNCB. As there are differences noted in potency and effects seen between PNCB (greater toxicity) and ONCB (lesser toxicity)(see below), we believe it appropriate to associate similarity of effects projected with MNCB with those of PNCB. This provides both a more conservative approach to assignment of effects as well as the most scientifically justifiable, as human experience and repeated dose testing in animals support closer analogy of response between MNCB and PNCB than between MNCB and ONCB.

Thus, we conclude that use of all available data in the Category approach, along with key studies with ONCB and PNCB, allows this HPV Endpoint to be completed without further unnecessary testing of MNCB.

Table 10. Summary of Developmental Toxicity and Reproduction Studies with Category Members

Chemical	Study Type/Species	Dosage	Observations	Conclusion
o-Chloronitro-benzene (ONCB) CAS NO. 88-73-3	Rat Teratology – Gavage 25 /group	150 mg/kg	Maternal Toxicity: 6/25 early deaths	no further investigation
		100 mg/kg	Maternal Toxicity: Body wt gain Food consump. 1 death; No terata, embryotox or fetotox	NOEL for Embryotoxicity, Fetotoxicity, Teratogenicity
		75 mg/kg	Maternal tox; Food consump. 1 death	
		25 mg/kg	No findings	NOEL for Maternal toxicity
o-Chloronitro-benzene (ONCB) CAS NO. 88-73-3	Mouse Continuous Breeding	160 mg/kg	Methem in FO & F1 FO (M/F) spleen wts F1(m) spleen and liver wts ; sem. Vesic.Wt F1 (Final litter) M/F pup wt.	NOEL – fertility Indices. Reproductive NOAEL was 320 mg/kg
		80 mg/kg	FO (M/F) spleen wts F1 (Final litter) M/F pup wt.	

		40 mg/kg	F1 (Final litter) female pup wt.	
m-Chloronitro-benzene (MNCB) CAS NO. 121-73-3	No studies found			
p-Chloronitro-benzene (PNCB) CAS No. 100-00-5	Rat Teratology – Gavage 25/group	45 mg/kg 15 mg/kg 5 mg/kg	Maternal toxicity: Body wt. Gain Spleen wt. Embryotoxicity: Resorptions Fetotoxicity: Fetal wts. Terata: skeletal Maternal toxicity: Body wt. Gain Spleen wt. No terata, embryo- or fetotoxicity No findings	 NOEL for teratogenicity, fetotoxicity and embryotoxicity Maternal toxicity NOEL
p-Chloronitro-benzene (PNCB) CAS No. 100-00-5	Rabbit Teratology - Gavage 18/group	125 mg/kg 75 mg/kg 25 mg/kg	Maternal Toxicity: Deaths (7/18) Physical changes Maternal toxicity: Physical changes No findings	NOEL for Terata, fetotoxicity, and embryotoxicity NOAEL for Maternal Toxicity Unequivocal NOEL for Maternal Toxicity
p-Chloronitro-benzene (PNCB) CAS No. 100-00-5	Two-generation Rat Gavage Reproduction Study 15 males/30 females per group in F0 and F1 generations	5 mg/kg 0.7 mg/kg	Parental toxicity: Histopathology consistent with methemoglobinemia F0/F1: all mating indices judged normal No findings	NOEL for all reproductive endpoints NOEL: Maternal & paternal toxicity

		0.1 mg/kg	No findings	
p-Chloronitro-benzene (PNCB) CAS No. 100-00-5	Mouse Continuous Breeding	250 mg/kg	Most animals visibly cyanotic FO-Fertility (after 1st litter) F1-spleen and liver wt ; estrus cycle F1 & F2 pup wt F2 pup survival and wts	
		125 mg/kg	FO-Fertility (after 1st litter) F1 & F2 pup wt	
		62.5 mg/kg	FO-Fertility (after 1st litter) F1 male pup wt	

In summary, as seen previously in sections dealing with acute and repeated dose testing for mammalian toxicity endpoints, PNCB has proven to produce the more significant comparative toxicity, hence the lower dosages used in the developmental toxicity studies listed. Albeit tested at lower dosages, only PNCB exhibited significant developmental toxicity in the comparative rat studies. Severe maternal toxicity, along with embryotoxicity, fetotoxicity and frank malformations were observed at the highest dosage tested. Only maternal toxicity and no embryotoxicity or fetotoxicity was observed at the mid dosage employed while the low dose selected was without treatment-related effect. As developmental effects were noted only at a dosage that produced significant maternal toxicity, PNCB is not considered to cause a primary effect on fetal development.

PNCB was toxic to rabbits in a developmental toxicity study (Nair et al, 1985). Frank maternal toxicity, including deaths, was observed at the highest dose tested, thus rendering determination of developmental toxicity impractical at this dosage level. There was no evidence of developmental toxicity observed at either of the two lower test levels used in this study.

ONCB, on the other hand, produced substantive maternal toxicity in rats at 100 mg/kg, but produced no evidence of embryotoxicity, fetotoxicity or teratogenicity even at this level.

PNCB produced no evidence of adverse reproductive performance, including mating, fertility and pregnancy, littering or pup survival and development, in a Two-Generation rat Reproduction study using a top dosage which produced significant maternal toxicity (increased spleen weight, anemia, elevated blood methemoglobin

levels) related to methemoglobinia following chronic dosing (Nair et al, 1989). PNCB, but not ONCB, affected reproductive outcomes in mice exposed during a series of continuous breeding cycles.

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US EPA, 1999a. Determining the adequacy of existing data. Guidance for the HPV Challenge Program (2/10/99).

US EPA, 1999b. The use of structure-activity relationships (SAR) in the High Production Volume Chemicals Challenge Program. OPPT, EPA.

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US EPA. 2002. ECOSAR model, v.0.99f.

Watanabe, T, Ishihara, N, Ikeda, M. 1976. Toxicity of and Biological Monitoring for 1,3-Diamino-2,4,6-trinitrobenzene and Other Nitro-amino Derivatives of Benzene and Chlorobenzene. Int. Arch. Occup. Environ. Health 37:157-168.

Zoeteman BCJ et al; Chemosphere 9: 231-49 (1980)

VII. ROBUST STUDY SUMMARIES
Appended

201-14392A

IUCLID

Data Set

Existing Chemical	:	ID: 100-00-5
CAS No.	:	100-00-5
EINECS Name	:	1-chloro-4-nitrobenzene
EC No.	:	202-809-6
TSCA Name	:	Benzene, 1-chloro-4-nitro-
Molecular Formula	:	C6H4ClNO2

Producer related part

Company	:	Solutia
Creation date	:	24.04.2004

Substance related part

Company	:	Revised by: Toxicology and Regulatory Affairs Freeburg IL 62243 618-539-5280
Creation date	:	24.04.2004
Status	:	
Memo	:	p-CNB
Printing date	:	01.06.2004
Revision date	:	
Date of last update	:	01.06.2004
Number of pages	:	1
Chapter (profile)	:	Chapter: 1.0.1, 1.2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.1, 3.3.2, 3.5, 4.1, 4.2, 4.3, 4.4, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.7, 5.8.1, 5.8.2
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: SIDS

2.1 MELTING POINT

Value	:	= 83.4 °C
Sublimation	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	
Method	:	not referenced
Test substance	:	p-Nitrochlorobenzene (CASNO 100-00-5)
Reliability	:	(2) valid with restrictions Citation from a reputable, universally accepted reference guide; value cited in the PNCB HSDB (2002).
Flag	:	Critical study for SIDS endpoint

25.04.2004 (1)

2.2 BOILING POINT

Value	:	= 242 °C at 1013.25 hPa
Decomposition	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	
Method	:	not reported
Remark	:	Listed as 242 deg. C @ 760 mm Hg.
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Citation from a reputable, universally accepted reference guide; value cited in the PNCB HSDB (2002).
Flag	:	Critical study for SIDS endpoint

31.05.2004 (1)

2.3 DENSITY

2.4 VAPOUR PRESSURE

Value	:	= .1253 hPa at 20 °C
Decomposition	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	
Remark	:	Reported as 0.094 mm Hg @ 20 deg. C.
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Cited as peer-reviewed in PNCB HSDB (2002).
Flag	:	Critical study for SIDS endpoint

31.05.2004 (2)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	= 2.39 at °C
pH value	:	
Method	:	other (measured)
Year	:	
GLP	:	no data
Test substance	:	
Method	:	Followed EPA methodology as defined in USEPA-600/4-79-032; Shake flask method using 6 replicates.
Test substance	:	p-Nitrochlorobenzene, CASNO 100-00-5
Reliability	:	(2) valid with restrictions Value derived from well accepted study design and consistent with other measured values reported in the literature (i.e. Hansch and Leo, 1995, SRC. Howard, 1990. Handbook of Environmental Fate and Exposure for Organic Chemicals. Lewis Pub.)
Flag	:	Critical study for SIDS endpoint

26.04.2004 (3)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 189.4 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	
Method	:	Published value
Remark	:	Support for this value comes from a published estimate for the material's water solubility, where the "group contribution method" was used to estimate a water solubility of 154 mg/L (para) Kuehne, R, RU Ebert, F, Kleint, G. Schmidt and G. Schuurmann. 1995. Group contribution methods to estimate water solubility of organic chemicals. Chemosphere 30(11):2061-2077.
Result	:	An experimental value of 189.4 mg/L was reported in this publication.
Test substance	:	p-Nitrochlorobenzene (CASNO 100-00-5)
Reliability	:	(2) valid with restrictions Published value
Flag	:	Critical study for SIDS endpoint

25.04.2004 (4)

3.1.1 PHOTODEGRADATION

Type	:	other
Light source	:	Xenon lamp
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	
Conc. of sensitizer	:	
Rate constant	:	cm^3/(molecule*sec)
Degradation	:	98 % after 5 hour(s)
Deg. product	:	yes
Method	:	other (measured)
Year	:	1979
GLP	:	no data
Test substance	:	other TS

Method :

Photochemical reactivity assay where 1 mL of PNCB in n-hexane was put in 1 L reaction vessel, followed by substitution of n-hexane vapor with air or nitrogen free from nitrogen oxides. PNCB was deposited in the reaction vessel, which corresponded to 1000 ul gas if vaporized and was irradiated at 25-30 deg. C for 5 hr with the Xenon lamp (ozone-less type, Ushio co.).Disappearance of TS measured by HPLC. Reaction by-products measured by GC-MASS.

Result :

Rate of disappearance was influenced by the intensity of light passing through either of two reaction vessels used in this experiment, i.e. pyrex and quartz. The rate of disappearance of PNCB in air free of nitrogen, when tested in pyrex and quartz vessels, respectively, was 4.1% and 96%. When PNCB was tested in nitrogen free of nitrogen oxides in pyrex and quartz vessels, respectively, disappearance rates were 7.1% and 98%. The single reaction by-product identified in air free from nitrogen oxides was 4-Chloro-2-nitrophenol while p-chlorophenol was the only by-product identified in nitrogen free from nitrogen oxides.

Test substance :

p-Chloronitrobenzene (CASNO 100-00-5)

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
31.05.2004 (5)

Type	:	other
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm^3
Rate constant	:	.0000000000001714 cm^3/(molecule*sec)
Degradation	:	50 % after 62.4 day(s)
Deg. product	:	
Method	:	other (calculated)

Year	:	2002
GLP	:	no
Test substance	:	other TS
Method	:	Used AOPWIN, v. 1.90 from EPIWIN, Syracuse Research Corp.
Result	:	Vapor phase of PNCB is susceptible to reaction with photochemically-produced hydroxyl (OH) radicals. The 2nd order rate constant for reaction with hydroxyl radicals was calculated as 0.1714E-12 cm3(molecule*sec). Based on 1.5E6 OH molecules/cm3 and assuming 12 hours of sunlight per day, the estimated photo-oxidation half-life is 62.4 days (~1500 hrs).
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Value obtained from EPA recommended estimation model.
Flag	:	Critical study for SIDS endpoint

31.05.2004 (6)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Degradation	:	< 50 % after 1 year at pH and °C
Method	:	Estimation on chemical principles Hydrolysis of chloronitrobenzenes to chlorophenols (hydrolysis of the nitro group) or to nitrophenols (hydrolysis of the chloro group) are both thermodynamically feasible as the enthalpy of reaction calculated from bond energies indicates hydrolysis to be a thermodynamically favored process. Hydrolysis of nitro group Delta H = 300 kJ/mol (aromatic nitro group) - 472 kJ/mol (phenol bond) Total enthalpy = - 172 kJ/mol Hydrolysis of chloro group Delta H = 407 kJ/mol (aromatic nitro group) - 472 kJ/mol (phenol bond) Total enthalpy = - 65 kJ/mol Although these reactions are thermodynamically favorable, the free energy of the transition state for this hydrolysis is so high that the reactions are generally not feasible (March's Advanced Organic Chemistry, fifth ed 2001 page 433).

It is known that special aromatic compounds, such as those with multiple electron withdrawing groups ortho and para to the halogen are potentially activated and can be substituted with difficulty. However, picryl chloride (with three nitro groups ortho or para to the chlorine) is stable in water at room temperature as it is transported with about 10% water to limit explosion potential. Thus, ortho and para CNB are anticipated to be hydrolysable under extreme conditions but are considered to have a hydrolytic half-life greater than one year under environmental conditions.

Bond energies from Lide, Handbook of Chemistry 84th edition 2003-2004 section 9

Result :

Considered to have a hydrolytic half-life greater than one year under environmental conditions.

Test substance :

p-Chloronitrobenzene [CAS No. 100-00-5]

Reliability : (2) valid with restrictions

Estimate by a reliable method

Flag : Critical study for SIDS endpoint

01.06.2004 (7)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media : other
Air : 9.52 % (Fugacity Model Level I)
Water : 28.5 % (Fugacity Model Level I)
Soil : 61.8 % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : .171 % (Fugacity Model Level II/III)
Method : other
Year :

Method :

Estimation using measured values from this dossier were incorporated into EPIWIN from Syracuse Research Corp., a methodology based on Meylan, 1993 as adopted by MacKay et al. 1996. Second Soil entry included estimation in Sediments. Values employed were : Mo. Wt = 157.56, vapor pressure of 0.094 mm Hg. Log Kow of 2.39, a melting point of 83 deg. C, and water solubility of 154 mg/L. Half lifes for air, water, soil and sediment were included as 1500 hr, 900 hr, 900 hr, and 3600 hr, respectively; emissions loading was 1000 kg/hr for each medium.

Remark :

Persistence Time was 506 hr.

Test substance :

p-Nitrochlorobenzene

Reliability : (2) valid with restrictions

Estimated values based on model recommended by US EPA.

Flag : Critical study for SIDS endpoint

31.05.2004 (6)

3.3.2 DISTRIBUTION

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	domestic sewage
Concentration	:	1 mg/l related to Test substance 10 mg/l related to Test substance
Contact time	:	
Degradation	:	34 - 66 (±) % after 24 hour(s)
Result	:	
Deg. product	:	
Method	:	other
Year	:	
GLP	:	no
Test substance	:	

Method :

Semi-Continuous Activated Sludge (SCAS) test conducted over 10-month period, in accordance with J Am Oil Chemists Society methods (JAOCS, 1965, 42:986 and JAOCS, 1965, 46:432). Inoculum was municipal waste treatment sludge. Feeding rate started at 1 mg/24-h and was raised in 1 mg increments to 5 mg over 28 days, and held at 5 mg/24-h for 4 months, then raised again to 10 mg/24-h. Twenty mL samples of mixed liquor (activated sludge + liquor) were taken 1 hr after each addition and at the end of the aeration cycle, via sidearm stopcock. The mixed liquor was extracted and analyzed via UV spectroscopy. Spike recovery experiments were 95.9 +/- 1.5%.

Result :

Average disappearance rate, days 75-120 (5 mg feed level, high aeration rate) was 33.9 +/- 2.9% over a 24-h cycle; over the next 60 days (same parameters) the disappearance rate was 30.7 +/- 9.4% over a 24-h cycle; over the last two weeks (10 mg feed level, low aeration), disappearance rate averaged 65.7 +/- 14.4% per 24-h cycle.

Test substance :

PNCB presumably as commercial grade with purity > 99%.

Reliability : (2) valid with restrictions

Study conducted prior to codification of GLPs but considered well documented. Methodology used has subsequently been incorporated into a standardized international test guideline for this study type.

Flag : Critical study for SIDS endpoint

25.04.2004 (8)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 1.8
LC50	:	= 6
Limit test	:	
Analytical monitoring	:	no
Method	:	other
Year	:	
GLP	:	yes
Test substance	:	other TS

Method :

Employed EPA methodology 660/3-75-009. Ten fish (ave. weight of 0.97 g and mean length of 40 mm), obtained from Trout Lodge, McMillin, WA, USA were tested in one of 5 test concentrations for up to 96-h. PNCB was administered in an acetone solution at concentrations of 1, 1.8, 3.2, 5.6 and 10 mg/L plus untreated and solvent control. Antimycin A was used as a positive control. Temperature was maintained at 12 +/- 1 deg. C. Tests were conducted in soft reconstituted deionized water, supplemented with 48 mg NaHCO3, 30 mg CaSO4, 30 mg MgSO4 and 2 mg KCL per liter. Fish were unfed 48 hr prior to testing and through the experimental period. Tests were conducted in 20-L glass vessels containing 15-L of solution. Dissolved oxygen was monitored to ensure the concentration did not fall below 2 mg/L before the end of the test. Water quality parameters such as pH, ammonia, and temperature were measured; no significant changes were observed during the test for these parameters. Estimation of LC50 and 95%CI were determined using EPA statistical procedures (probit analysis).

Remark :

Supporting ECOSAR Calculations are:

```
SMILES : c1c(CL)ccc(N(=O)(=O))c1
CHEM   : para-Chloronitrobenzene
CAS Num: 100-00-5
ChemID1:
ChemID2:
ChemID3:
MOL FOR: C6 H4 CL1 N1 O2
MOL WT : 157.56
Log Kow: 2.39  (User entered)
Melt Pt: 83.40 deg C
Wat Sol: 189 mg/L  (measured)

ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics


                                        Predicted
ECOSAR Class       Organism    Duration  End Pt mg/L (ppm)
```

```
================= ==========  ========  ====  ==========
Neutral Organic SAR : Fish        14-day  LC50  96.772
(Baseline Toxicity)

Neutral Organics   : Fish         96-hr   LC50  50.216
Neutral Organics   : Fish         14-day  LC50  96.772
Neutral Organics   : Daphnid      48-hr   LC50  55.276
Neutral Organics   : Green Algae  96-hr   EC50  35.342
Neutral Organics   : Fish         30-day  ChV    6.889
Neutral Organics   : Daphnid      16-day  EC50   3.362
Neutral Organics   : Green Algae  96-hr   ChV    4.428
Neutral Organics   : Fish  (SW)   96-hr   LC50  13.891
Neutral Organics   : Mysid Shrimp 96-hr   LC50  10.963
Neutral Organics   : Earthworm    14-day  LC50 735.052 *

 Note:  * = asterick designates: Chemical may not be soluble
        enough to measure this predicted effect.
         Fish and daphnid acute toxicity log Kow cutoff: 5.0
         Green algal EC50 toxicity log Kow cutoff: 6.4
         Chronic toxicity log Kow cutoff: 8.0
         MW cutoff: 1000
```

Calculated 2004, by Toxicology and Regulatory Affairs, Freeburg IL using measured Kow

Result :

The 96-h LC50 (95%CL) = 6.0 (4.8-7.6) mg/L.; the 48-h LC50 (95%CL) = 7.5 (6.1-9.2) mg/L; the 24-h LC50 (95% CL) = 8.8 (no CL calc.) mg/L. No deaths were observed up to 3.2 mg/L through 96 hrs. At the 5.6 mg/L level the following % mortality was reported at 24, 48 and 96-h: 0%, 10%, 50%. At 10 mg/L, mortality reached 70%, 90%, and 90% at 24, 48 and 96-h. Toxicity as exhibited by surfacing was seen at concentrations of 3.2 mg/L and higher beginning 24 hr after treatment while loss of equilibrium also was seen at 10 mg/L at all three time points. Dissolved oxygen ranged between 9.2-7.1 mg/L, pH between 7.2-7.6 and total nitrogen (NH3) of <0.1 - 0.3 mg/L.

Test substance :

PNCB (CASNO 100-00-5) with purity of > 99% (listed as 99.21%).

Reliability : (2) valid with restrictions

Well documented study which followed regulatory guidance for study conduct. LC50 value identical to that reported for guppies (Deneer et al. 1987. Aquat Toxicol 10:115) and similar to LC50 of 8.3 for bluegill sunfish (Solutia study no. AB-80-316)

Flag : Critical study for SIDS endpoint

31.05.2004 (9)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= 3.2
EC50	:	= 10
Analytical monitoring	:	no
Method	:	other
Year	:	
GLP	:	yes
Test substance	:	

Method :

Employed EPA methodology 660/3-75-009. Ten < 24-h old D. magna Straus (lab culture) were tested at 23 deg C in a series of three replicates per test concentration. PNCB in dimethyl formamide was tested at 6.25, 12.5, 25, 50 and 100 mg/L plus untreated control and solvent control. Morbidity and mortality were checked daily. Tests were conducted in 250-mL beakers containing 200 mL of solution. Well water from St Peter, MO, USA was used. Daphnids received no food 48-h prior to treatment. Water quality was measured to record dissolved oxygen, pH, alkalinity, hardness and temperature. Determination of EC50 and 95%CL were made using EPA statistical procedures (Steven, CE 1976. ASTM STP 634).

Remark :

Supporting ECOSAR Calculations are:

```
SMILES : c1c(CL)ccc(N(=O)(=O))c1
CHEM   : para-Chloronitrobenzene
CAS Num: 100-00-5
ChemID1:
ChemID2:
ChemID3:
MOL FOR: C6 H4 CL1 N1 O2
MOL WT : 157.56
Log Kow: 2.39  (User entered)
Melt Pt: 83.40 deg C
Wat Sol: 189 mg/L  (measured)

ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics
                                                    Predicted
ECOSAR Class       Organism    Duration  End Pt mg/L (ppm)
================== ==========  ========  ====  ==========
Neutral Organic SAR : Fish        14-day  LC50  96.772
(Baseline Toxicity)

Neutral Organics   : Fish         96-hr   LC50  50.216
Neutral Organics   : Fish         14-day  LC50  96.772
Neutral Organics   : Daphnid      48-hr   LC50  55.276
Neutral Organics   : Green Algae  96-hr   EC50  35.342
Neutral Organics   : Fish         30-day  ChV    6.889
Neutral Organics   : Daphnid      16-day  EC50   3.362
```

```
Neutral Organics   : Green Algae  96-hr   ChV    4.428
Neutral Organics   : Fish  (SW)   96-hr   LC50  13.891
Neutral Organics   : Mysid Shrimp 96-hr   LC50  10.963
Neutral Organics   : Earthworm    14-day  LC50 735.052 *

 Note:  * = asterick designates: Chemical may not be soluble
        enough to measure this predicted effect.
         Fish and daphnid acute toxicity log Kow cutoff: 5.0
         Green algal EC50 toxicity log Kow cutoff: 6.4
         Chronic toxicity log Kow cutoff: 8.0
         MW cutoff: 1000
```

Calculated 2004, by Toxicology and Regulatory Affairs, Freeburg IL using measured Kow

Result :

48-h EC50 (95% CL) = 11.1 mg/L (8.9-13.3); 24-h EC50 = 18.8 (16.9-21.1) mg/L. Dissolved oxygen ranged between 8.1-8.4 mg/L, pH was 7.0-8.1, alkalinity was 266-340 mg/L and hardness ranged between 226-318 mg/L. Temperature remained constant at 23 deg. C. The NOEC was < 6.25 mg/L. Per cent deaths seen at 24 and 48 hr respectively were : none in control or solvent control, 6.25 mg/L - 3%, 23%; 12.5 mg/L - 7%, 50%, 25 mg/L - 83%, 90%, 50 mg/L - 100% at both time points and at 100 mg/L - 100% deaths at both time points.

Test substance :

PNCB (CASNO 100-00-5) with purity of > 99%.

Reliability : (2) valid with restrictions

Well conducted study following regulatory accepted test guidelines. Solutia study (AB-80-317) using similar design and employing two replicates per dose resulted in 48-h EC50 of 10 (9-12) mg/L.

Flag : Critical study for SIDS endpoint

31.05.2004 (10)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Scenedesmus subspicatus (Algae)
Endpoint : biomass
Exposure period : 48 hour(s)
Unit : mg/l
EC10 : = 2.2
EC50 : = 8
growth EC10 : = 4.9
growth EC50 : = 16
Method : other: DIN 38 412
Year : 1988
GLP : no data
Test substance :

Method :

DIN 38412, Part 9 - The green alga S. subspicatus (Strain 8681 SAG) was used to conduct a modified cell multiplication inhibition test. A stock solution of the test substance was prepared in double-distilled water and diluted to prepare a series of test concentrations ranging from 0.80-100 mg/L.

The test was conducted in capped 250 ml Erlenmeyer flasks.
Eight (8) replicates of each concentration in defined algal growth media were tested.
Flasks were inoculated with the cell suspension (cell concentration of 10E5 cells/ml in each flask), placed on a white surface, protected from sunlight, shaken daily, and exposed to constant artificial lighting. The temperature was maintained at 24 +/- 1 deg C. and the relative humidity was 50%. A control group (8 replicates) was tested concurrently. On each measurement day, 50 ml were collected from each of two flasks from each test concentration or the control. The extinction value of the monochromatic radiation (578 nm wavelength) of the cell suspension was determined for each test concentration and the control.
Samples were collected and measurements were made at the beginning of the test and after 24 and 48 hrs. Biomass determination was based on measurement of optical density (turbidity). EC values were determined graphically by regression analysis.
The test used static conditions. Initial pH was adjusted to 8.0, final pH was measured but not reported.

Remark :

Supporting ECOSAR Calculations are:

```
SMILES : c1c(CL)ccc(N(=O)(=O))c1
CHEM   : para-Chloronitrobenzene
CAS Num: 100-00-5
ChemID1:
ChemID2:
ChemID3:
MOL FOR: C6 H4 CL1 N1 O2
MOL WT : 157.56
Log Kow: 2.39  (User entered)
Melt Pt: 83.40 deg C
Wat Sol: 189 mg/L  (measured)

ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics
```

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organic SAR (Baseline Toxicity)	Fish	14-day	LC50	96.772
Neutral Organics	Fish	96-hr	LC50	50.216
Neutral Organics	Fish	14-day	LC50	96.772
Neutral Organics	Daphnid	48-hr	LC50	55.276
Neutral Organics	Green Algae	96-hr	EC50	35.342
Neutral Organics	Fish	30-day	ChV	6.889
Neutral Organics	Daphnid	16-day	EC50	3.362
Neutral Organics	Green Algae	96-hr	ChV	4.428
Neutral Organics	Fish (SW)	96-hr	LC50	13.891
Neutral Organics	Mysid Shrimp	96-hr	LC50	10.963
Neutral Organics	Earthworm	14-day	LC50	735.052 *

```
 Note:  * = asterick designates: Chemical may not be soluble
        enough to measure this predicted effect.
         Fish and daphnid acute toxicity log Kow cutoff: 5.0
         Green algal EC50 toxicity log Kow cutoff: 6.4
         Chronic toxicity log Kow cutoff: 8.0
         MW cutoff: 1000
```

Calculated 2004, by Toxicology and Regulatory Affairs, Freeburg IL using measured Kow

Result :

Mean measured values of control group at 48 hrs were extinction value - 0.068; Biomass - 3.6 x 10E5 cells/ml. Results of the cell multiplication inhibition test of PNCB were: 48-h Biomass EC10 = 2.2 mg/L; 48-h Biomass EC50 = 8.0 mg/L. The 48-h average specific growth rate EC10 = 4.9 mg/l; 48-h average specific growth rate EC50 = 16 mg/L.

Test substance :

p-Nitrochlorobenzene (CASNO 100-00-5), purity unspecified.

Reliability : (2) valid with restrictions

Small deviations from standard study design, including shorter duration used (48 vs 72 h), and limited information presented on each test concentration at each measurement point.

Flag : Critical study for SIDS endpoint

31.05.2004 (11)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 530 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	20
Vehicle	:	other
Doses	:	
Method	:	other
Year	:	
GLP	:	no
Test substance	:	

Method :

Methodology similar to OECD # 401, except with fewer animals; PNCB was administered by gavage in 10% corn oil to groups of 5 mixed sex SD rats at dosages of 398, 501, 631 and 794 mg/kg. Animals were observed for signs of toxicity and death daily for 14 days. Body weights were recorded on study day 0 and weekly thereafter. Animals dying and all survivors to d14 were necropsied. Food and water were given ad libitum and temp., humidity and light were controlled. LD50 and CI were calculated by the method of deBeer, J. Pharmacol Experiment Ther 86:1.

Result :

LD50=530 mg/kg with CI of 480-590 mg/kg; Incidence of deaths observed at each dose group were: 1/5 @398 mg/kg, 2/5 @ 501 mg/kg, 4/5 @ 631 mg/kg, and 5/5 @ 794 mg/kg. Deaths occurred during study days 1-5, with most occurring during days 1-3. Clinical signs of toxicity observed included: increased weakness, slight tremors, ocular discharge. Necropsy of the viscera in decedents resulted in identification of lung hyperemia and discoloration of the liver, spleen and kidneys. Viscera of survivors (14 days) appeared normal.

Test substance :

p-Chloronitrobenzene (CASNO 100-00-5)

Reliability : (2) valid with restrictions

Study conducted prior to codification of OECD guideline 401 or inception of US GLPs (1979). Fewer animals used than stipulated in 401. Test results are highly consistent with 17 other rat OLD50 values ranging betwen 294-830 mg/kg as found in the ECB IUCLID PNCB, 2000.

Flag : Critical study for SIDS endpoint

31.05.2004 (12)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	inhalation
Exposure period	:	6 hr/day
Frequency of treatm.	:	5 days per week for 13 weeks
Post exposure period	:	
Doses	:	0, 1.5, 3, 6, 12 and 24 ppm
Control group	:	yes
NOAEL	:	< 1.5 ppm
LOAEL	:	= 1.5 ppm
Method	:	OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year	:	1989
GLP	:	yes
Test substance	:	other TS

Method :

Groups of 10 male and 10 female F-344 rats were exposed in whole body stainless steel and glass chambers to vapors containing 0, 1.5, 3, 6, 12 or 24 ppm PNCB for 6 hr/d, 5 days per week, for 13 weeks. Vapor was generated by transfer of bulk PNCB into a flask and attached to a vapor generator with a rotary evaporation system. The resulting vapor was forced into a condenser and temperature maintained by circulating oil. Generator output and flow were automatically controlled. Chamber monitoring was performed using a GC/EC system. Low volatility of PNCB limited the maximum exposure vapor concentrations to the top level used in this study. Animals were individually caged, food and water administered ad libitum, and a 12 hr light:dark cycle employed. All animals were assessed for morbidity and mortality daily and weekly examined for clinical toxicity and recording of body weights. At termination of the study (13 weeks) all animals were necropsied and a full set of over 40 tissues and organs were examined microscopically for all high dose and control animals; target organs were examined for animals from lower dose groups. Organ weights and relative weights were assessed for all animals after 13 weeks of testing and included the following organs: heart, kidney, lung, liver, spleen, testis and thymus. The following hematology parameters were assessed on study day 1 (Methemoglobin only), 4, 23, and at 13 weeks from all rats from each study group: HCT, HGB, RBC, RETIC, MCV, MCH, MCHC, PLAT, WBC, MET, and WBC differentials. Similary, the following clinical chemistry parameters were measured from all rats at similar time points as hematology: BUN, CREAT, TPROT, ALB, GLOB, ALT, AP, CK, SDH, and bile acids. Williams parametric multiple comparison procedure was employed to statistically assess group-wise comparison of organ and body weights. Shirley's test for nonparametric analysis was used for clinical chemistry and hematology assessments. $P<0.05$ and <0.01 were used in all cases.

Remark :

Sperm morphology and vaginal cytology evaluations were performed on rats exposed to 0, 6, 12, or 24 ppm PNCB. Male rats exposed to 24 ppm exhibited significantly lower left epididymal, cauda epididymal, and testis weights and lower spermatid heads/testis, spermatid counts and spermatozoal concentrations than control rats; estrous cycle length was decreased in all groups of PNCB-exposed females.

Result :

Mean concentrations in all test chambers were between 99-100% of target concentrations. No treatment related deaths, obvious clinical signs of toxicity, or effects on body weight were observed at any dose level. Hematology findings were consistent with methemoglobinemia and macrocytic (increased MCV) and hyperchromic (MCHC increase) hemolytic anemia seen at all test levels. Compensatory hematopoietic cell proliferation was present and considerable hemosiderin deposition observed microscopically, and produced a pattern of effects observed with other MET-forming agents. Following are the various statistically elevated/depressed effects noted at each dose level: At 1.5 ppm = increased MET, normocytic RBC (F only) and decreases in HCT, HGB, RBC, ALT (M only), renal hyaline droplet formation (males only), splenic congestion and hemosiderosis; at 3 ppm = increased MET, RETIC, normocytic RBC and bile acids (M only), and decreases in HCT, HGB, RBC, ALT, (M only), AP (F only), marked increase in spleen wt and mild liver wt (F only), renal hyaline droplets (M only), bone marrow hematopoietic cell proliferaton, Hardarian gland inflammation, congestion and hemosiderosis of the spleen along with hematopoietic cell proliferation and capsular fibrosis and hemosiderosis of the liver Kupfer cells (F only); at 6 ppm = increases in RETIC, MET, n-RBC, bile acids (M only), SDH (F only), MVC, spleen and liver weights and decreases in HCT,RBC, ALT, AP, TPROT, GLOB, and renal hyaline droplet formation (M only), bone marrow and splenic hematopoietic cell proliferation, Hardarian gland inflammation, splenic congestion, hemosiderosis and capsular fibrosis of the liver; at 12 ppm = decreases in HCT, HGB, RBC,AP, GLOB, ALT, TPROT and increases in MET, RETIC, n-RBC, SDH and bile acids, marked increases in spleen weights and mild increases in liver, heart and thymus weights, renal hyaline droplet formation (M only), bone marrow and splenic hematopoietic cell proliferation, Hardarian gland cell proliferaton, splenic congestion, hemosiderosis, and capsular fibrosis and hemosiderosis and histiocytic hyperplasia of the liver; at 24 ppm = increases in MET, RETIC, MCV, n-RBC, HGB, SDH, bile acids and decreases in HCT, HGB, RBC, AP, GLOB, ALT, TPROT, organ weight increases of the spleen, liver, heart, thymus and decreased testes weight, renal hyaline droplet formation (M only), bone marrow and splenic hematopoietic cell proliferation, Hardarian cell proliferation, splenic congestion and capsular fibrosis, hemosiderosis of the spleen and liver, histiocytic liver hyperplasia and testicular atrophy

Test substance	:	PNCB determined to be > 97 % pure.
Reliability	:	(1) valid without restriction Well documented study consistent with OECD test guideline 413
Flag	:	Critical study for SIDS endpoint

31.05.2004 (13)

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	6 hr/day
Frequency of treatm.	:	5 days/week for 4 weeks
Post exposure period	:	
Doses	:	0, 5, 15, and 45 mg/m3 (equivalent to 0.78, 2.3 and 7 ppm)
Control group	:	yes
NOAEL	:	< 5 mg/m³
LOAEL	:	= 5 mg/m³
Method	:	OECD Guide-line 412 "Repeated Dose Inhalation Toxicity: 28-day or 14-day Study"
Year	:	1982
GLP	:	yes
Test substance	:	other TS

Method :

Groups of 10 male and 10 female SD rats were exposed via whole body in stainless steel and glass inhalation chambers to airborne concentrations of 0, 5, 15 or 45 mg/m3 PNCB for 6 hr/day, 5 days/week for 4 weeks. PNCB was mixed with a solvent and fed into a spray atomizer through which dry air was passed. Test material flow into test chambers was controlled using a fluid metering pump. Concentrations of PNCB were determined at least 3X daily using UV spectrophotometer; particle size distribution was determined throughout the study. Parameters monitored in this study included daily morbidity and mortality checks, weekly detailed clinical observations, and body weights. Hematology parameters (HGB, RBC, HCT, RETIC, MET, clotting time, RBC morph. and total and differential leukocytes) and clinical chemistries (BUN, SGPT, AP, GLU, ALB, TPROT, GLOB, K, CL, CA, PHOS) were analyzed at day 0 and just prior to termination (MET also analyzed after 2 weeks of testing) for 10 rats/sex/group. Ophthalmoscopic exams were conducted on all rats prior to study start and at termination. Organ (brain, testes, ovaries, heart, kidneys, pituitary, liver, lungs, spleen) weights and weight ratios were recorded at terminal sacrifice for all rats on test. Microscopic examination of over 40 tissues and organs were performed on all rats from the high dose and control groups at the end of the study. Spleens of all low and mid dose animals were also examined microscopically. Gormori's stain was used to semiquantitate the degree of hemosiderosis. A Bartletts' test was performed on study data to determine the degree of equality of variances (Snedecor and Cochran) followed by

Dunnet's test for parametric parameters and the Kruskal-Wallis test along with Dunn's Summed Rank test for nonparametric parameter analysis. P <0.05 was used in all cases.

Result :

Cumulative mean analytical exposure concentrations were 5, 16 and 45 mg/m3. Particle size distribution of the generated atmospheres established that PNCB was introduced as a vapor, rather than as an aerosol. No mortalities were observed in treated groups and mean body weights of PNCB-treated animals were similar to control values. Clinical signs of toxicity observed included: cyanosis of the conjuctivae, nasal areas and entire body in all three groups, with incidence increasing with dose. Other than a dark red appearance, no ocular abnormalities related to treatment were observed. Rats at all test levels exhibited slight reductions in HGB, HCT, RBC at one or both study intervals. Animals in the mid and high dose groups also exhibited an increase in the incidence of poikilocytosis and polychromia at the interim bleeding. MET showed a dose-related increase with levels approximating 2-8X controls. An increase in leukocytes was attributed to the abberant inclusion of reticulocytes in the automatic counting procedure for white blood cells. Small increases in GLU and reduced PHOS levels were seen in HD females only. Statistically elevated spleen and liver weights were seen in HD males and females (and rel. liver wts in mid dose females). An increased incidence of congestion, extramedullary hematopoiesis and hemosiderosis of the spleen was observed in male and female rats exposed to 45 mg/m3 and iron-positive pigmentation (hemosiderosis) in spleens of rats from the mid and low dose.

Test substance :

p-Chlorobenzene, greater than > 99 % pure

Reliability : (1) valid without restriction

Provided as Supplemental information as a longer-term study, conducted by the same exposure route, has been selected as the Key study for this HPV Endpoint; this study meets OECD Test Guidance 412 and was conducted under GLPs.

Flag : Critical study for SIDS endpoint

31.05.2004 (14)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	Salmonella typhimurium strains TA100, TA98, TA1535, TA1537
Test concentration	:	10, 4, 3, 1.5, 1.3, 1, 0.3, 0.2, 0.04, and 0.01 mg/plate
Cycotoxic concentr.	:	3 mg/plate
Metabolic activation	:	with and without
Result	:	positive
Method	:	OECD Guide-line 471
Year	:	
GLP	:	yes
Test substance	:	

Method :

Method used was plate incorporation assay based on Ames test methods consistent with OECD 471. All tests were run in duplicate and three plates were assayed at each dosage for each run both with and without metabolic activation. The S-9 liver homogenates were prepared from male SD rats and CD-1 mice given Arochlor 1254. All tester strains were obtained from Dr. B. Ames. Sterile DMSO was used as the solvent and a solvent control was employed of 20 uL/plate DMSO. Positive controls used were: 2-aminoanthracene, 9-aminoacridine, benzo(a)pyrene, NaNo2 and 2-nitrofluorene. A positive response was determined upon observation of a statistically significant dose-response increase in revertant colonies. Bartlett's test was used for pairwise comparison to controls and dose response determined using regression analysis for log-log straight lines; P<0.01 was used.

Result :

A definitive positive response was observed with TA1535 without metabolic activation, with some indication that TA1535 with metabolic activation was marginally positive.

Test substance :

p-Chloronitrobenzene, CASNO 100-00-5, purity greater than 99%

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
25.04.2004 (15)

Type	:	Cytogenetic assay
System of testing	:	Chinese Hamster Ovary Cell in vitro assay
Test concentration	:	50 to 5000 ug/mL
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: NTP
Year	:	
GLP	:	yes
Test substance	:	

Method :

Study conducted according to NTP study design, testing involved 3 separate tests (2 with S9 and 3 without S9 fraction added) SD male rat Arochlor 1254-induced liver homogenate was used. Cell cultures were handled to prevent

photolysis of Brdu-substituted DNA. Each test consisted of concurrent solvent and positive controls and at least 3 dose levels. Cells were incubated in McCoy's 5A medium with test agent ranging between 10.5-19 hrs, colcemid added and incubated for an additional 2 hrs and harvested/processed. 100 first-division metaphase cells were scored blind from prepared slides for each dose level. Classes of aberrations were recorded and included simple, complex and other abnormalities. Statistical analysis (Armitage trend test; Margolin multiple comparison test) were conducted on both the dose-response curve and individual dose points; significance was determined as $P<0.05$ for single data points and $P<0.015$ for trend.

Result :

Initial trials run at harvest times of 10.5 hr w and w/o S9 were negative. A follow up trial w/o S9 conducted at a higher dose level (700, 800 and 900 ug/ml) and incubated for 19 hrs (because PNCB induced cell cycle delay in the earlier study) resulted in an increase in aberrant cells only at 900 ug/plate; A repeat of this study at levels of 500, 600 and 700 ug/plate resulted in a dose related increase only at the top dose used. Repeat of the metabolic activation trial using a longer period to harvest (19 hr) produced an increase in aberrant cells.

Test substance :

4-Chloronitrobenzene, CASNO 100-00-5, from MC/B Chemical Co, Lot D11F12, practical grade, >99% pure by independent analysis.

Reliability : (2) valid with restrictions

Provided as Supplemental information as an in vivo cytogenetics study has been used to fulfill this HPV endpoint. Study considered reliable.

Flag : Critical study for SIDS endpoint

25.04.2004 (16)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : rat
Sex :
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : once
Doses : 30, 100 and 300 mg/kg
Result : negative
Method : OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"
Year : 1983
GLP : yes
Test substance :

Method :

Dose levels selected based on pilot study which produced 1/4 deaths @ 400 mg/kg and 4/4 deaths @ 600 mg/kg. Five rats/sex/time period were administered PNCB in corn oil by gavage. Metaphase cells were collected from rat bone marrow

(femur) at harvest times of 6, 12 and 24 hrs after treatment from 5 rats/sex. Colchicine was administered 2 hr prior to sacrifice to arrest cells in c-metaphase. Marrow was exposed to hypotonic solution and fixed, cells and slides prepared and stained. All slides were coded before reading. Positive (cyclophosphamide) and negative (corn oil) controls were used for comparative purposes. Mitotic index was calculated based on counting of at least 500 slides and all breaks, deletions, translocations and other changes recorded. Breaks or aberrations between treated vs control groups were compared by Chi-square analysis. P <0.05 was used.

Result :

Rats dosed with 100 and 300 mg/kg PNCB exhibited signs of cyanosis; animals given 300 mg/kg lost weight between time of dosing and sacrifice. No significant differences were observed in the frequency of breaks or aberrations between PNCB-treated and control groups at any of the three time points measured.

Test substance :

p-Chloronitrobenzene, CASNO 100-00-5, purity > 99%.

Reliability : (2) valid with restrictions

Time points measured did not include a period beyond 24 hr, but sufficient cells in metaphase were obtained at this time point that it was determined that there was no need to extend the sampling period.

Flag : Critical study for SIDS endpoint

01.06.2004 (17)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : Two generation study
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : F0 & F1 Adults-premating through litter weaning (Fo) and postweaning (F1)
Frequency of treatm. : daily (7d/wk) gavage
Premating exposure period
Male : FO- 14 weeks; F1- 18 weeks
Female : FO-14 weeks; F1- 18 weeks
Duration of test : FO M/F - 167d; F1 M/F- 219d
No. of generation studies :
Doses : 0, 0.1, 0.7 and 5.0 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL parental : = .7 mg/kg bw
NOAEL F1 offspring : = 5 mg/kg bw
NOAEL F2 offspring : = 5 mg/kg bw
Method :
Year :
GLP : yes

Method :

Test material was administered to groups of 15M and 30F rats (vehicle control group also included) in corn oil to F0 and F1 generations during a premating (14 wks for F0 and 18 wks for F1) growth period, and through the ensuing mating, gestation and lactation intervals (1 litter/generation). F1 rats continued on treatment during a post-weaning period of 30d. Dosing concentrations analyzed by GC weekly for the first week of the study and monthly thereafter for accuracy Body weights were recorded weekly for F0 and F1M. For F0 and F1 F wts were recorded weekly through the growth period and up to mating, then resumed after mating until sacrifice. Food consumption was recorded weekly for F0 and F1 M from study start up to mating, then resumed after mating through study term. Food consumption for adult females F0 and F1 was recorded weekly through the growth period and again after weaning of litters. Cageside observations for morbidity and mortality were made weekly, as well as daily observations of clinical signs. Temperature, humidity and light-dark cycles were controlled. F0 adults were sacrificed following weaning of the F1 litters and given a gross postmortem examination; reproductive tissues (testes, epididymides, seminal vesicles) were evaluated histopathologically for all control and high dose males. Adult F1 M and F rats were sacrificed following completion of a post-weaning treatment interval, given a gross necropsy. A full histopathological examination of over 40 tissues and organs (including gonads) was performed on 10 randomly selected F1 adult animals/sex/group. Pups delivered to F0 and F1 females were evaluated for growth, survival and external irregularities during lactation days 0, 4, 14 and 21. F1 pups not selected for the adult generation were sacrificed and given a gross postmortem exam. Tissues were evaluated histopathologically (~40 tissues/organs) from 5/sex/group of F1 weanlings and F2 weanlings. Body weights and changes, food consumption, gestation length and number of offspring were analyzed using ANOVA techniques followed by Dunnet's Test for parametric parameters and Kruskal-Willis test followed by Dunn's Rank Sum for nonparametric analysis. Mortality and pregnancy rates, fetal and mating indices and pup survival were analyzed using Chi-square, followed by Fisher Exact test and Armitage's test for linear trend. The level of significance was reported at both the 5% and 1% levels.

Remark :

Slight decreases in male and female fertility indices, as well as testicular effects seen in 3 HD male rats in the FO generation are considered spurrious findings, unrelated to treatment. No such effects were noted in the F1 generation, which were exposed over a considerably longer dosing period. Likewise, no testicular effects were observed in a group of 50 male rats exposed to 5 mg/kg/d PNCB by gavage for 24 months, a dosing regimen similarly used here, albeit for substantively longer than the 14 weeks rats in the HD group in this study were dosed.

Result : Dosing solutions were confirmed analytically as accurately prepared. No treatment-related mortalities could be affirmed in this study, although several gavage-related deaths occurred sporadically. Mean body weights and weight gains of all FO male groups were similar; FO females exhibited slightly, but not statistically lower, body weights at all treatment levels. This finding was considered unrelated to treatment as there was no dose-response effect noted. Food consumption values were similar between treated and control FO males and females, except for HD females which consumed slightly, but not statistically signficantly, more food through the first 6 weeks of the study. The mating index (no. mating/total given opportunity to mate) were similar for all FO Males. The mating index for FO Females was 86.7, 80, 71.4 and 71.4%, respectively, from control through HD group; as all these values were within the historical control range for this indice in this laboratory, these findings were considered unrelated to treatment. No statistical differences were seen in either pregnancy rate or male fertility index between PNCB-treated and control animals from the FO generation. Three HD male rats in the FO parental generation were found to have testicular degeneration upon microscopic examination. FO dams treated with PNCB during gestation and lactation exhibited mean body weights and length of gestation indices comparable to control levels. The number of live and dead pups at birth and pup weights durng lacation of pups from FO dams were unaffected by PNCB dosing. Pup survival in the HD group was slightly, but statistically significantly lower than the control group. This finding was related to the complete loss of two litters in this group, a phenomenon experienced within the test lab on an infrequent, but not unusual, basis. Thus, this finding was judged unrelated to PNCB treatment. No compound-related gross postmortem changes were observed in the FO adults or F1 weanlings. F1 generation: No treatment-related effects were seen in any test group for survival, mean body weights and gains, and food consumption during mating, gestation and lactation. No treatment-related effects were observed during the gross postmortem evaluation of F1 adults. An increase in extramedullary hematopoiesis and brown pigmentation of reticuloendothelial cells of both sexes of the HD treated groups were observed following histological examination of F1 animals.All three PNCB-treated groups of F1 females exhibited a slightly (but not statistically) lower mating index than the control group; however, no dose-response was evident and the number of pregnant females in all groups, control and treated, were similar. Thus, this observation was not considered treatment-related. Male (FO1) mating and fertility indices were unaffected by treatment. Litter and pup survival indices in groups of F2 generation animals were comparable between all treated and control groups.Similarly, pup weights were unaffected by treatment at all test levels. No evidence of toxicity was observed during the gross postmortem evaluation of F2 pups and no compound-related changes were observed during histological examination of tissues in F1 and F2 weanling pups.

Test substance :

p-Nitrochlorobenzene, CASNO 100-00-5, Monsanto lot KM06 328, purity 99.43% (0.47% ortho isomer and 0.1% meta isomer)

Conclusion :

Systemic toxicity was observed in high-dose males and females evidenced as methemoglobinemia; thus, the NOAEL for systemic toxicity was considered to be 0.7 mg/kg-day

No adverse effects on reproductive endpoints were observed in any group and the reproductive NOAEL is considered to be 5 mg/kg-day.

Reliability : (1) valid without restriction

Well documented GLP study meeting OECD Test Guideline 416.

Flag : Critical study for SIDS endpoint

31.05.2004 (18)

Type : other
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 105 days
Frequency of treatm. : daily, 7 days per week for 7 days prior to cohousing and 98 days of cohousing
Premating exposure period
Male : 7 days
Female : 7 days
Duration of test : 98 days of continuous breeding
No. of generation studies :
Doses : 0, 62.5, 125 and 250 mg/kg/d
Control group : yes, concurrent vehicle
Method : other
Year :
GLP : yes
Test substance :

Method :

Standard Continuous breeding protocol designed by NTP and published as Lamb, 1985. J. Amer. Coll. Toxicol. 4:163-171. Based on 2-week toxicity test to establish dose levels, animals are individually housed for 7 days, then cohoused in breeding pairs for 98 days, and allowed to propagate. During this period the following indices are recorded: clinical signs of toxicity, mortality, parental body weight and average consumption of water during representative weeks, fertility (e.g. no. of pairs producing a litter/number of breeding pairs), the no. of litters per pair, the no. live pups/litter, % pups born alive, sex ration of pups and pup body weights after birth). The last litter born during the holding period (5 weeks) following the breeding period is reared until weaning after which treatment of the F1 animals was initiated and these animals used for assessment of second generation fertility. For this phase, siblings were cohoused until sexual maturity, when 20 non-sibling males and females per treatment group were cohabitated for 7 days and then housed singly through delivery. Endpoints for this

mating trial were the same as for the F0 generation. At termination of F0 and F1 generations, animals were necropsied and evaluations made for organ weights (ovaries or testes and epididymides from 5 per group, control and HD), body weights, epididymal sperm motility, sperm morphology, sperm count and estrual cyclicity. Methemoglobin measurements and spleen weights were recorded for both the F0 and F1 generations. Proportional data were assessed statistically using the Armitage trend test, with each dose group compared to control using a chi-square analysis. Absolute body and organ weights were compared using Shirley's or Dunn's test while dose related trends were identified by Jonckheere's test. Vaginal cytology was analyzed using an analysis of variance described by Morrison to test for simultaneous equality. A p value of <0.05 or <0.01 was used.

The following reproductive organs were weighed and examined microscopically as appropriate.
- Ovaries
-Testes
-Epididymis
-Seminal vesicles
-Prostate

Result :

Fertility of mice dosed with PNCB decreased progressively with the duration of dosing and with increasing dose and being statistically different from controls at the high dose level. Most mice exposed to 250 mg/kg were cyanotic. Spleen and liver weights of F1 PNCB-treated mice reportedly were significantly greater than those of the controls. Survival and body weights of F1 (final litter) and F2 pups were significantly decreased at 250 mg/kg and at 125 mg/kg (F1 only).

NOAEL's are difficult to specify in this study and may be less than 62.5 mg/kg-day for doth systemic effects and repeoductive effects.

Test substance :

p-Nitrochlorobenzene, CASNO 100-00-5, purity 97%

Reliability : (1) valid without restriction

GLP study, peer reviewed and followed an established study design.

Flag : Critical study for SIDS endpoint

27.04.2004 (13)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	once per day
Frequency of treatm.	:	gestation days 6 through19
Duration of test	:	rats sacrificed on gestation day 20
Doses	:	5, 15 and 45 mg/kg/day

Control group	:	yes
NOAEL maternal tox.	:	< 5 mg/kg bw
NOAEL teratogen.	:	= 15 mg/kg bw
NOAEL Fetotoxicity	:	= 15 mg/kg bw
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	1980
GLP	:	yes
Test substance	:	other TS

Method :

Groups of 24 mated female rats were dosed daily by gavage (test material dissolved/suspended in corn oil) during gestation days 6-19. All rats were observed for mortality and abnormal behavior twice daily from gestation day 0 through day 20, at which time all animals were sacrificed and maternal spleen weights recorded. Detailed physical exams for signs of toxicity were recorded on study days 0, 6, 10, 15 and 20. Maternal body weights were recorded at several intervals throughout the study. At sacrifice the uterine horns were examined for implantation sites, resoptions and the number of viable or non-viable fetuses. The number of corpora lutea were also recorded. The sex and weights of all live fetuses were recorded and all fetuses were examined for external abnormalities. One-half of the fetuses per litter were examined for skeletal malformations while the other half were examined for internal anomalies. The following statistical analyses were performed: For interval data (body wts, wt changes, reproductive data) Bartlett's test was used to determine equality of variance and ANOVA and Dunnett's test used for parametric data while the Kruskal-Wallis test and Summed Rank test used for nonparametric data (Snedecor and Cochran). For Incidence data, i.e. mortality rates, % and incidence of variations and malformations comparisons were made using the Chi-square contingency table and the 2X2 Fisher Exact test employing the Bonferroni inequality estimate; linear trend was evaluated using the Armitage test. Comparisons were made using the litter as the comparative entity. Both the 5% and 10% level of statistical significance were reported for each parameter.

Result :

Maternal toxicity (reduced body weight gain during the treatment period and increased spleen weights), fetotoxicity (increased no. resorptions/litter), embryotoxicity (increased no. fetuses with unossified sternebrae, incompletely ossified cervical and vertebral transverse processes) and fetal skeletal (predominantly angulated ribs) malformations were observed at the 45 mg/kg dosage level. At 15 mg/kg, similar maternal toxicity but no fetotoxic/embryotoxic or teratogenic responses were observed. At 5 mg/kg, only a slight increase in spleen weight was observed in maternal animals.

Test substance :

p-Nitrochlorobenzene, CASNO 100-00-5, Monsanto lot KM06 328, purity 99.43% (0.47% ortho isomer and 0.1% meta isomer)

Conclusion :

PNCB produced teratogenic effects only at dosages which

produced significant maternal toxicity.

Reliability : (1) valid without restriction

This study meets OECD Test Guideline 414 and was conducted under GLPs.

Flag : Critical study for SIDS endpoint

27.04.2004 (19)

Species	:	rabbit
Sex	:	female
Strain	:	New Zealand white
Route of admin.	:	gavage
Exposure period	:	gestation days 7 through 19
Frequency of treatm.	:	once daily
Duration of test	:	animals sacrificed on gestation day 30
Doses	:	0, 5, 15, 40 mg/kg
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 15
NOAEL teratogen.	:	= 15
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	
GLP	:	yes
Test substance	:	

Method :

Groups of 18 mated female NZ white rabbits were administered PNCB in corn oil (constant volume of 2 ml/kg) in corn oil at PNCB concentrations of 0 (vehicle control), 5, 15, and 40 mg/kg on gestation days 7-19. Animals were evaluated for detailed signs of toxicity on test days 0, 7, 10, 15, 19 and 30; body weights were recorded on test days 0, 7, 19 and 30. Daily observations were made for morbidity and mortality. Food and water were administered ad libitum and a 12 light:dark cycle was employed. Temperature and humidity were controled. All animals were examined externally and 1/2 were evaluated for soft tissue malformations and the other 1/2 for skeletal findings. The following statistical analyses were performed: For interval data (body wts, wt changes, reproductive data) Bartlett's test was used to determine equality of variance and ANOVA and Dunnett's test used for parametric data while the Kruskal-Wallis test and Summed Rank test used for nonparametric data (Snedecor and Cochran). For Incidence data, i.e. mortality rates, % and incidence of variations and malformations comparisons were made using the Chi-square contingency table and the 2X2 Fisher Exact test employing the Bonferroni inequality estimate; linear trend was evaluated using the Armitage test. Comparisons were made using the litter as the comparative entity. Both the 5% and 10% level of statistical significance were reported for each parameter.

Result : Mortality so high at 40 mg/kg level that this study group was terminated without additional data collection. 15 mg/kg and 5 mg/kg- no effects on survival or maternal body wts, no treatment-related effects in uterine implantation data, fetal wts or sexing data. No statistically significant differences seen in skeletal malformations between treated and control groups nor was there any treatment-related

increase in the incidence of external or soft tissue findings.

Test substance :

p-Nitrochlorobenzene, CASNO 100-00-5, Monsanto lot KM06 328, purity 99.43% (0.47% ortho isomer and 0.1% meta isomer)

Reliability : (1) valid without restriction

Well conducted study following GLP guidance and OECD study design. Limited due to excessive no. of deaths at the high dose group which disallowed any developmental toxicity information to be obtained from this study group.

Flag : Critical study for SIDS endpoint

27.04.2004 (20)

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