Chemical Name: 2-Pyrrolidone
CASRN: 616-45-5
Submitter: BPPB Consortium

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:

201-14223

To: Oppt.ncic@epamail.epa.gov
cc: Jane Vergnes <JVergnes@ispcorp.com>, Christopher Bradlee <bradlec@basf-corp.com>

Subject: HPV Submission CASNO 616-45-5

Attached is the HPV submission for 2-Pyrrolidone CASNO 616-45-5. There are three attachments in pdf format:

1. Cover letter

2. Test plan

3. Robust summaries

This submission is made on behalf of the BPPD Consortium [redacted]

Please call or email me if you have any difficulty receiving or opening the submission.

Elmer Rauckman PhD DABT

rauckman@toxicsolutions.com

618-539-5280

616-45-5-CL.pdf 616-45-5-TP.pdf 616-45-5-RS.pdf

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December 30, 2002

Christine Todd Whitman
US Environmental Protection Agency
PO Box 1473
Merrifield VA 22116

Re: Submission of 2-Pyrrolidone (CASNO 616-45-5) Documents

Via Electronic Submission to Oppt.ncic@epa.gov

Registered with EPA as:
BPPB Consortium, **Registration Number**

Dear Administrator Whitman;

On behalf of the 2-Pyrrolidone Consortium, I am submitting the attached test plan and robust summaries for 2-Pyrrolidone (CASNO 616-45-5), submitted under the United States Environmental Protection Agency's High Production Volume Chemical Challenge Program. This submission consists of a test plan and a set of robust summaries for this material.

The Consortium members sponsoring this submission are

- BASF Corporation
- International Specialty Products

This document is being submitted in electronic format (Adobe Acrobat pdf files). If you require additional information or have problems with the electronic document please contact me as a representative of the Consortium by phone (618-539-5280) or email (erauckman@charter.net).

Sincerely,

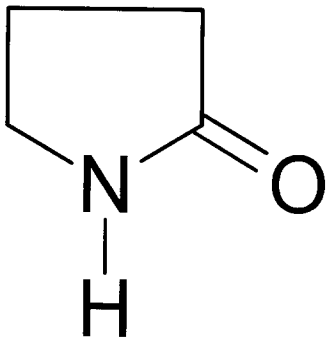
Elmer Rauckman, PhD, DABT
Consulting Toxicologist

Attachments:
Testing Plan 616-45-5-TP.pdf
Robust Summaries 616-45-5-RS.pdf

CC: BASF
ISP

201-14223A

2-Pyrrolidone



CAS Number 616-45-5

U.S. EPA HPV Challenge Program Submission

December 30, 2002

Submitted by:

2-Pyrrolidone Consortium

Prepared by:
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Table of Contents

Executive Overview

2-Pyrrolidone, CAS no. 616-45-5, is a cyclic amide prepared primarily from butyrolactone. It is a clear liquid with an unpleasant ammonia-like odor and a freezing point of 25° C. It has low volatility (boiling point 245 °C and vapor pressure of 0.013 hPa @ 25° C) and is miscible with water and most organic solvents. Its most extensive use is as a chemical intermediate but it is also used as a high-boiling solvent.

In the environment, based on physicochemical and experimental data, 2-Pyrrolidone will not bioaccumulate (Log $K_{o/w}$ = -0.71) and will distribute primarily to water where it will be subject to limited volatilization and rapid biodegradation. It is expected to react rapidly with atmospheric hydroxyl radicals with a half-life of about 11 hours. The toxicity of Propargyl alcohol to aquatic species is very low, with an LC_{50} for freshwater fish greater than 4600 mg/L and daphnia greater than 1000 mg/L.

The oral LD_{50} of 2-Pyrrolidone is very high with values of 8000 and greater than 5000 mg/kg being reported. Exposure of rats to saturated vapor for 8 hours did not produce any adverse effects and the dermal LD50 in rabbits is greater than 2000 mg/kg.

A modern subchronic drinking water study of 2-Pyrrolidone showed low repeated-dose toxicity with a 90-day NOAEL of 2400 ppm and a LOAEL of 7200 ppm in drinking water. The kidneys many have been affected but no target organs were identified by histopathological examination.

Adequate *in vitro* tests of genetic toxicity for 2-Pyrrolidone are available. A *Salmonella typhimurium* reverse mutation assay shows lack of mutagenic activity in the presence or absence of metabolic activation and a guideline cytogenetics study using human lymphocytes displayed a lack of genotoxicity activity in the presence or absence of metabolic activation.

Developmental toxicity has been investigated using an OECD 414 Guideline study. The results of this investigation conducted in rats by oral gavage at 0, 190, 600 or 1900 mg/kg-day indicate that 2-P affects the conceptus only at doses that exceed the maternally toxic level. The developmental NOAEL was found to be 600 mg/kg-day while the maternal NOAEL was 190 mg/kg-day.

The combination of the negative developmental toxicity study with a robust subchronic study in which specific damage to reproductive organs was not observed fulfills the current requirement for reproductive toxicity information.

It is concluded that the available information adequately fills all the data elements of the HPV. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, conduct of additional similar studies would not add significantly to our understanding of this material's hazard.

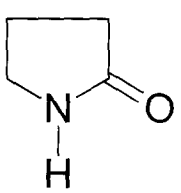
Testing Plan and Rationale

Testing Plan in Tabular Format

CAS Number 616-45-5 2-Pyrrolidone	Information Available?	OECD Study ?	GLP Study?	Supporting Information?	Estimation Method?	Acceptable?	Testing Recommended?	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	N	N	Y	N	
Boiling Point	Y	N	N	N	N	Y	N	
Vapor Pressure	Y	N	N	Y	N	Y	N	
Partition Coefficient	Y	Y	N	Y	N	Y	N	
Water Solubility	Y	N	N	Y	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	N	Y	Y	N	
Water Stability	Y	N	N	Y	Y	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	Y	N	N	Y	N	Y	N	
Ecotoxicity								
96-Hour Fish	Y	Y	N	Y	N	Y	N	
48-Hour Invertebrate	Y	Y	N	Y	N	Y	N	
72-Hour Algae	Y	Y	N	Y	N	Y	N	
Toxicity								
Acute	Y	N	N	Y	N	Y	N	
Repeated Dose	Y	Y	Y	N	N	Y	N	
Genetic Toxicology *in vitro*	Y	N	Y	Y	N	Y	N	
Genetic Toxicology *in vivo*	Y	N	Y	Y	N	Y	N	
Reproductive	Y	N	N	Y	N	Y	N	
Developmental	Y	Y	Y	Y	N	Y	N	

Introduction

2-Pyrrolidone, CAS no. 616-45-5, is a cyclic amide prepared primarily from butyrolactone by a Reppe process (1). It is a clear liquid (above 25° C) with an unpleasant ammonia-like odor. It has low volatility and is miscible with water and most organic solvents. Its most extensive uses are as an intermediate in the manufacture of N-methylpyrrolidone, vinylpyrrolidone, polyvinylpyrrolidone and polypyrrolidone with over 95% of the 2-Pyrrolidone production going into vinylpyrrolidone (2). It is used as a high-boiling solvent in petroleum processing and acrylonitrile manufacture. It also finds application as a solvent for polymers, sorbitol, glycerol, iodine and sugars. Some is used as a plasticizer and coalescing agent for polymer emulsion coatings such as floor polishes. Another application is as humectant and co-solvent for digital printing inks. It's exceptional solvent properties make it very useful for the solubilization of complex organic material in water. Although it is an excellent solvent, the somewhat labile proton on the nitrogen limits its applications as an aprotic solvent. Its structure is shown below:
2-Pyrrolidone is also known as:



- 4-Aminobutyric acid lactam
- Gamma-aminobutyric lactam
- Gamma-aminobutyrolactam
- Butanoic acid, 4-amino-, lactam
- Butyrolactam
- Gamma-butyrolactam
- 2-Ketopyrrolidine
- 2-Oxopyrrolidine
- 2-Pyrol
- Apha-pyrrolidinone

The chemical and physical properties of 2-Pyrrolidone make it a unique solvent for certain applications and a useful chemical intermediate. There are several reports in the open literature of its utility as a skin-penetration enhancer with potential applications in transdermal drug delivery. This property and potential application seems

to be a function of the physicochemical properties of this solvent and not a specific chemical reactive property. Another use in the pharmaceutical industry is in the production of pyrrolidone nootropics including piracetam (2).

Exposure in industrial applications is limited by process controls, protective equipment, a very low vapor pressure and excellent warning properties due to its objectionable odor. No occupational exposure level set by a governmental agency could be located for 2-Pyrrolidone. Use as a humectant and co-solvent in digital inks may result in a low-level of inhalation exposure by consumers limited by the very low quantities of inks used by digital printing devices.

Several physicochemical, fate and toxicity studies have been conducted on 2-Pyrrolidone. These studies are briefly reviewed in this testing rationale document, which also describes how these studies meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (USEPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries or given as shorter summaries using the IUCLID format. The available data set satisfactorily fulfills the data requirements for the EPA HPV Program. The majority of data elements are filled by high-reliability studies on 2-Pyrrolidone. Where direct data are not available or data are sparse, surrogates and estimations are used to fill the data element. This is encouraged by the U.S. EPA and other regulatory authorities to avoid unnecessary testing and animal usage.

Physicochemical Data

Physicochemical data for 2-Pyrrolidone are available from the literature and manufacturer's information.

Table 1: Physicochemical Properties of 2-Pyrrolidone

Melting Point	25° C (3)
Boiling Point	245° C @ 1010 hPa (4)
Vapor Pressure	0.013 hPa @ 25° C (5)
Partition Coefficient	Log $K_{o/w}$ = -0.71 (6)
Water Solubility	Soluble in all proportions (7)

These properties indicate that above 25° C, 2-Pyrrolidone is slightly volatile liquid with high water solubility. The value of the partition coefficient suggests that 2-Pyrrolidone will partition preferentially into water and, therefore, has little potential for bioaccumulation.

Recommendation: No additional physicochemical studies are recommended. The available data fill the HPV required data elements.

Environmental Fate and Pathways

Biodegradation potential has been determined using a Zahn Wellens test. In this DOC removal test, DOC was 80% eliminated after 5 days of incubation (8). Although this only definitively shows "inherent biodegradability" the speed of removal and completeness (99% at 9 days) suggest that this material is easily biodegraded by non-adapted bacteria. Using BIOWIN 4.00, it can be estimated that 2-Pyrrolidone is readily biodegradable with quantitative estimates suggesting a high likelihood that it should be considered "readily biodegradable (9). Furthermore, the analog and surrogate compound, N-Methyl-2-pyrrolidone (NMP) has been demonstrated to be readily biodegradable in the MITI test (10). Comparative estimation using BIOWIN 4.00 suggests that NMP is likely to be slightly more resistant to aerobic biodegradation than 2-Pyrrolidone, although NMP still is indicated by BIOWIN to be readily biodegradable. The information that NMP biodegradation is correctly predicted as readily biodegradable by BIOWIN, and the strong structural similarity between the two compounds, validates the BIOWIN estimate for 2-Pyrrolidone.

Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The program produced a estimated rate constant of 11.9 E-12 cm^3/molecule-sec. Using the default atmospheric hydroxyl radical concentration in APOWIN and the estimated rate constant for reaction of 2-Pyrrolidone with hydroxyl radical, the estimated half-life of 2-Pyrrolidone vapor in air is approximately 10.75 hours (see accompanying robust summary).

Water stability has not been quantitatively determined for 2-Pyrrolidone. Quantitative stability determinations (e.g. OECD 111) are considered unnecessary for compounds containing only non-hydrolysable groups, as the SIDS manual states that consideration should be given to using an estimation method. There is no evidence available that 2-Pyrrolidone is unstable in water, although it has a potentially hydrolysable amide group, amides are considered resistant to hydrolysis at environmental pH values and require strong base or acid to accomplish hydrolysis. Vollhardt states: "Amides are the least reactive of the carboxylic derivatives, mainly because of the extra resonance capacity of the nitrogen lone electron pair. As a consequence, their nucleophilic addition-eliminations require relatively harsh conditions. For example, hydrolysis occurs only on prolonged heating in strongly acidic or basic water"(11). The HYDOWIN program recognized this when an estimate of hydrolysis was attempted. The HYDROWIN output was that the compound had an amide group and the hydrolysis rate was extremely slow, the HYDROWIN program estimated the half-life in water greater than one year (12). This estimated is confirmed by the review of Harris, who notes that the mean hydrolytic half-life for a series of amides is in the range of 300 years (13). In addition, this is a cyclic amide in a 5-membered ring, which is generally the ring size showing the least strain and, hence making ring opening a less favored occurrence increasing resistance to hydrolysis.

Theoretical Distribution (Fugacity) of 2-Pyrrolidone in the environment was estimated using the MacKay EQC level III model with standard defaults in EPIWIN v 3.05 but using the measured vapor pressure of 0.013 hPa and the measured log $K_{o/w}$ (14). The results for distribution using a model calculated $K_{o/c}$ (adsorption coefficient based on organic carbon content) of 0.0799 and equal initial distribution to air, water and soil are:

- Air 0.4 %
- Water 46.5 %
- Soil 53.0 %
- Sediment 0.08 %

Recommendation: No additional fate studies are recommended. The available data fill the HPV required elements.

Ecotoxicity

A recent GLP guideline (OECD 203) study of acute fish toxicity using measured concentrations of 2-Pyrrolidone is available demonstrating low hazard to zebra fish after 96 hours of exposure. The test material stability in the dilution water with fish was very good over the 96-hour period. Daphnia studies indicate an EC_{50} greater than 1000 mg/L in one test, greater than 500 mg/L in another guideline-like study and a report of an EC_{50} values less than 20 mg/L. Although experimental data give differing results, the weight of evidence indicates a low aquatic hazard. Other invertebrates, specifically, flatworms and snails, showed no effects in limit tests at 112 mg/L. Algae growth inhibition, according to a guideline study, has an EC_{50} of about 84 mg/L after 96-hours. These values with references are shown in the table. ECOSAR estimates, using the neutral organic model, are also given in the table below for comparison. In addition, a bacterial growth inhibition test using *Pseudomonas putida* resulted in an EC_{50} of 9368 mg/L, with lower concentrations showing stimulation of bacterial growth (15).

Table 2: Comparative Aquatic Toxicity of 2-Pyrrolidone

	Reported Values	ECOSAR Prediction
Fish, 96 hour LC_{50}	> 4600 mg/L (16)	9566 mg/L*
Daphnia, 48 hour EC_{50}	> 500 mg/L (17) > 1000 mg/L (18) = 13.2 mg/L (19)	8733 mg/L*
Algae, 96 hour EC_{50}	= 84 mg/L (20)	4777 mg/L*

* Estimated using ECOSAR (21)

Unvalidated, but multiple, study results reported in IUCLID 2000 (22) indicate that the analog 1–methyl–2–pyrrolidone has low acute toxicity to fish, invertebrates and algae (short-term LC_{50} or EC_{50} values >500 mg/L). This lends support to the higher values for the LC_{50} and EC_{50} values of 2-Pyrrolidone that have been reported. The reason some investigations have found higher degrees of toxicity is unknown but a reasonable speculation would be that the samples tested were contaminated with more toxic agents. For example, it is known that γ-Butyrolactone which is one of the primary starting materials for 2-Pyrrolidone is more toxic to fish and daphnids. Likewise, aliphatic amines, which are potential side products from 2-Pyrrolidone manufacture, typically have LC and EC_{50} values in a range where contamination of a sample might result in a low EC_{50}.

Recommendation: No additional ecotoxicity studies are recommended. The available data fill the HPV required endpoints. Although experimental data give differing results, the weight of evidence indicates low aquatic hazard. This information coupled with the information that 2-Pyrrolidone is biodegraded easily in the environment and has a low log $K_{o/w}$ constant reduce the concern level for potential environmental hazard. Conduct of additional studies would not add significantly to our understanding of this material's toxicity and it is recommended that no additional ecotoxicity studies be conducted.

Health Effects

Acute Toxicity

Oral Exposure

Multiple determinations of the oral LD_{50} of 2-Pyrrolidone have been reported (23) and the studies universally indicate a low order of acute oral toxicity for this material. Two robust summaries have been prepared from BASF study reports. One indicted an LD_{50} of approximately 8000 mg/kg-bw (24) and the other was a limit test at 5000 mg/kg-bw in which there were no mortalities or adverse clinical signs except for transient loss in male body weights (25).

Inhalation Exposure

It has been reported that there were no deaths when rats were exposed to saturated vapor of 2-Pyrrolidone for 8 hours (26). The actual concentration was not measured but based on the vapor pressure at 30°C the vapor concentration is calculated to be in the range of 15-20 ppm.

Dermal Exposure

A guideline (OECD 402) limit study has indicated that the dermal LD_{50} of 2-Pyrrolidone in rabbits is greater than 2000 mg/kg-bw (27).

Recommendation: No additional acute toxicity studies are recommended. The available data fill the HPV required endpoints for acute toxicity. Although the available studies do not meet the requirements of the current OECD guidelines in all cases, the weight of evidence shows that the oral and dermal toxicity is very low. Likewise, the limited study of acute saturated vapor inhalation provides important and scientifically defensible information about vapor toxicity. Conduct of additional studies would not add significantly to our understanding of this material's toxicity and it is recommended that no additional acute toxicity studies be conducted.

Repeat Dose Toxicity

Oral Exposure

A guideline-glp 90-day study in rats has been conducted. In this study, 2-Pyrrolidone was administered to groups of 10 male and 10 female Wistar rats at doses of 0; 600; 2,400; 7,200 and 15,000 ppm in the drinking water over a period of 3 months (28). No animals died nor were any adverse clinical signs of exposure reported. In the high-dose group, food and water consumption, and body-weight gain were reduced for males and females; kidney weights for males and females were increased; other minor treatment related effects were in prolonged prothrombin times and decreased serum protein, globulins, creatinine and triglycerides. At 7,200 ppm, water

consumption was reduced in rats of each sex; food consumption and body weight gain were reduced only for females; kidney weights for males were increased; other minor treatment related effects were in and decreased serum total protein for females and decreased creatinine in both sexes. The 2,400 ppm dose was a NOAEL. Gross pathology, organ weight determination and full histopathology were conducted on all animals. No treatment-related histopathologic effects were observed.

Recommendation: No additional repeated-dose studies are recommended. The available data conducted by OECD Guidelines and under GLP fill the HPV required endpoint for repeated-dose toxicity.

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points: generally one sensitive to point mutation and one sensitive to chromosomal aberrations. In the case of this material, adequate tests have been conducted that cover both of these endpoints.

Genetic Toxicology in vitro

Adequate *in vitro* tests of genetic toxicity for 2-Pyrrolidone are available. A *Salmonella typhimurium* reverse mutation assay shows lack of mutagenic activity in the presence or absence of metabolic activation (29). Likewise, a guideline cytogenetics study using human lymphocytes displayed a lack of genotoxicity activity in the presence or absence of metabolic activation (30).

Genetic Toxicology in vivo

Mammalian genotoxicity was assessed *in vivo* using the Mouse Micronucleus Test. In this OECD-Guideline-474 study, a single i.p. dose of 2-Pyrrolidone did not result in an increase in normochromatic erythrocytes containing micronuclei. It was concluded that the test material did not show genotoxic activity in this system (31).

Recommendation: The SIDS requirement for genetic testing has been met as assays sensitive to both point mutation and to clastogenic effects have been conducted using acceptable protocols. No additional genotoxicity testing is recommended.

Reproductive Toxicity

The combination of the negative developmental toxicity study (32) with a robust subchronic study (28) showing that, even at systemically toxic doses, there is no specific damage to reproductive organs of males or females, fulfills the current requirement for reproductive toxicity information.

Recommendation: No additional reproductive testing is recommended, as the available data are sufficient to assess the reproductive toxicity of this material.

Developmental Toxicity

A modern OECD 414 Guideline study has been conducted with 2-Pyrrolidone. The results of this investigation conducted in rats by oral gavage at 0, 190, 600 or 1900 mg/kg-day indicate that 2-Pyrrolidone is embryotoxic at doses that exceed the maternally toxic level. The developmental NOAEL was found to be 600 mg/kg-day while the maternal NOAEL was 190 mg/kg-day. Even at the maximum dose level of 1900 mg/kg-day the developmental toxicity was not severe (32). This result is supported by an older single-dose-level teratology study at about 1900 mg/kg-day in the same strain of rat by oral gavage. In this study, 25 presumed-pregnant dams were treated from day 6 to 15 of gestation. Fetuses were delivered by Caesarean section on GD-20 and examined for external, visceral and skeletal abnormalities. No differences were reported between the control and treated animals (33). A mouse teratology study using i.p. injection has also been conducted. Some degree of developmental toxicity was reported in this study but the effect was considered due to stress on the animals from the i.p. injections (34). The proposed explanation is consistent with mouse physiology; moreover, the route of exposure is inappropriate in a consideration of hazard or risk assessment.

Taken together, the weight of evidence from these developmental toxicity studies indicate a low developmental toxicity hazard for 2-Pyrrolidone.

Recommendation: No additional developmental toxicity testing is required as the available data are sufficient to assess the developmental toxicity of this material.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, it is concluded that the available information fills all of the requirements for physicochemical parameters, fate information, aquatic toxicity and mammalian toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, taken together the information provided a reliable hazard assessment. Conduct of additional studies would not add significantly to our understanding of this material's toxicity.

References

1 O'Neil, MJ (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Thirteenth edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001

2 Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VHC Verlag GmbH, 2002

3 Flick, E.W. (ed.). Industrial Solvents Handbook 4 th ed. Noyes Data Corporation., Park Ridge, NJ., 1991. 918, as cited in Hazardous Substance Data Base, NLM, Revison of 8-6-2002

4 Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996. 1378

5 Daubert, T.E. and Danner, R.P. Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. Design Institute For Physical Property Data, American Institute Of Chemical Engineers. Hemisphere Pub. Corp., New York, NY., 5 Vol, 1997

6 BASF AG, Analytisches Labor; Unpublished Study (J.Nr.129300/04 vom 14.06.88)

7 Riddick, J.A.; Bunger, W.B.; and Sakano, T.K. Organic Solvents: Physical Properties And Methods Of Purification. Techniques Of Chemistry. 4th Ed. New York, NY: Wiley-Interscience. 2: Pp.1325, 1986 (as cited in CIS 4-2002)

8 BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung (Pyrrolidon dest., 1977) see robust summary.

9 BIOWIN 4.00 SRC, See Robust Summary for details of method and results of modeling.

10 Chem Inspect Test Inst; Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan; Published by Japan Chemical Industry Ecology-Toxicology & Information Center. ISBN 4-89074-101-1 p. 5-5 (1992)

11 Vollhardt, K. "Organic Chemistry" WH Freeman and Co, New York, 1987, p 815.

12 HYDROWIN v1.67, Syracuse Research Corporation, Syracuse NY, available through the U.S. EPA.

13 J.C. Harris in Lyman W, Reehl, W and Rosenblat, D. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washingotn D.C. 1990, page 7-6

14 EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

15 BASF AG: Labor Okologie, unpublished study, 28.06.88

16 BASF AG: Abt. Toxikologie, unpublished report, (92/14), 01.08.1995

17 BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0701/88)

18 Submission to U.S. EPA: Raw data for ecotoxicity information on 2-Pyrrolidinone (CAS Reg No 616-45-5), with cover letter dated 01/29/86 Source: EPA/OTS; Doc #FYI-OTS-0794-1152 Submitted by Eastman Kodak Company

19 Perry, C.M., Smith,S.B. Toxicity of Six Heterocyclic Nitrogen Compounds to Daphnia pulex. Bull. Environ. Contam. Toxicol.41, 604-608, (1988)

20 BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0701/88, Fa.Noack)

21 ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

22 ECB IUCLID 2000, 1–methyl–2–pyrrolidone, 19-Feb-2000, ECB

23 ECB IUCLID (2000) document for 616-45-5 2-Pyrrolidone 18-FEB-2000, ECB

24 BASF AG, Abteilung. Toxikologie, unveroeffentlichte Untersuchung,(XI/407), 07.11.1961

25 BASF AG, Abteilung Toxikologie; unveroeffentliche Untersuchungen (79/409), 09.04.1981

26 BASF AG: Abt. Toxikologie, unveroeffentlichte Untersuchung,(XI/407), 07.11.1961

27 MB Research Laboratories Inc project number MB-92-1432 Sponsored by International Specialty Products, 4/29/1992.

28 BASF AG, Report of the Subchronic oral toxicity with 2-Pyrrolidone in Wistar rats, 3-month drinking water, Project No. 52S0014/92038 June 4, 1998

29 Jagannath, D.R., Mutagenicity Test on 2-Pyrrolidone in the Ames Salmonella/Microsome Reverse Mutation Assay, Final Report, Hazleton Labs, GAF Sponsor April 24, 1987.

30 BASF AG, Abt. Toxikologie, unpublished study report (86/286), 26.11.1987

31 BASF AG, Abteilung Toxikologie; unpublished report. Cytogenetic Study In Vivo of Pyrrolidon-2 in Mice, Micronucleus test. (92/1491), 28.06.93

32 Bio-Research Laboratories Inc, An Oral Teratoloty Study of 2-Pyrrolidone in the Rat. Project # 83880, Dec. 19, 1990 Sponsored by GAF Chemicals and BASF AG

33 BASF AG, Abt. Toxikologie, unveroeffentlichte Untersuchung (XIX/421), 04.08.1971

34 BASF AG, Abteilung Toxikologie; unveroeffentliche Untersuchung (XIX/421), 29.05.1970

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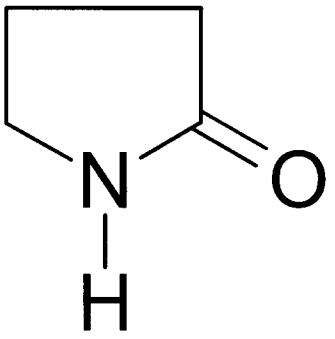
2-Pyrrolidone



CAS Number 616-45-5

Existing Chemical	:	ID: 616-45-5
CAS No.	:	616-45-5
EINECS Name	:	2-pyrrolidone
EC No.	:	210-483-1
TSCA Name	:	2-Pyrrolidinone
Molecular Formula	:	C4H7NO

Producer related part

Company	:	Toxicology and Regulatory Affairs
Creation date	:	06.10.2002

Substance related part

Company	:	Toxicology and Regulatory Affairs
Creation date	:	06.10.2002
Status	:	
Memo	:	
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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: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1. General Information

Id 616-45-5
Date 31.12.2002

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	lead organisation
Name	:	Toxicology and Regulatory Affairs
Contact person	:	Elmer Rauckman PhD DABT
Date	:	
Street	:	1201 Anise Court
Town	:	62243 Freeburg, IL
Country	:	United States
Phone	:	618-539-5280
Telefax	:	618-539-5394
Telex	:	
Cedex	:	
Email	:	rauckman@toxicsolutions.com
Homepage	:	toxicsolutions.com
Remark	:	Participating Members of Consortium BASF Corporation International Specialty Products

31.12.2002

1.2 SYNONYMS AND TRADENAMES

2-Ketopyrrolidine

08.12.2002

2-Oxopyrrolidine

08.12.2002

2-Pyrol

08.12.2002

4-Aminobutyric acid lactam

08.12.2002

Apha-pyrrolidinone

08.12.2002

Butanoic acid, 4-amino-, lactam

08.12.2002

Butyrolactam

08.12.2002

Gamma-aminobutyric lactam

1. General Information

Id 616-45-5
Date 31.12.2002

08.12.2002

Gamma-butyrolactam

08.12.2002

2. Physico-Chemical Data

Id 616-45-5
Date 31.12.2002

2.1 MELTING POINT

Value	:	= 25 °C
Test substance	:	2-Pyrrolidone CAS No. 616-45-5
Reliability	:	(2) valid with restrictions 2 Handbook Value
Flag	:	Critical study for SIDS endpoint
06.10.2002		(20)

2.2 BOILING POINT

Value	:	= 245 °C at 1010 hPa
Decomposition	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	
Test substance	:	CAS No. 616-45-5 2-Pyrrolidone
Reliability	:	(2) valid with restrictions Handbook values are assigned 2
Flag	:	Critical study for SIDS endpoint
06.10.2002		(15)

2.3 DENSITY

Type	:	density
Value	:	= 1.116 g/cm³ at 25 °C
Method	:	
Year	:	
GLP	:	no data
Test substance	:	
Test substance	:	CAS No. 616-45-5 2-Pyrrolidone
Reliability	:	(2) valid with restrictions 2 Handbook Value
Flag	:	Critical study for SIDS endpoint
06.10.2002		(15)

2.4 VAPOUR PRESSURE

Value	:	= .013 hPa at 25 °C
Decomposition	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	

2. Physico-Chemical Data

Id 616-45-5
Date 31.12.2002

Remark	:	Given in reference as 0.00949 mm. Converted to hPa by multiplying by 1.33 hPa/mm Supported by IUCLID 2000 value of 0.04 hPa at 20 C as referenced in BASF AG, Sicherheitsdatenblatt Pyrrolidon dest. (28.06.1993)
Reliability	:	(2) valid with restrictions 2 Handbook Value
Flag	:	Critical study for SIDS endpoint

31.12.2002 (17)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	= -.71 at 25 °C
pH value	:	
Method	:	OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year	:	
GLP	:	no data
Test substance	:	
Method	:	Approximately 25 ml each of water and 1-octanol were mixed in a shake flask with 0.063, 0.137 or 0.166 grams of test substance in three separate trials at 25 deg C. After separation of the layers, the test substance was determined in quadruplicate in each phase with using gas chromatography. The mean P(OW) values for each of the three trials were 0.193, 0.193 and 0.206. These values were averaged and the log was determined to give a mean Low K0/w of -0.71
Remark	:	SRC Physical Properties Data Base lists result 0r -0.85 as published by Sasaki,H et al. (1991). EPIWIN, Log Kow (KOWWIN v1.66 estimate) = -0.32 based on smiles structure.
Test substance	:	2-Pyrrolidone CAS No. 616-45-5
Reliability	:	(1) valid without restriction 1, Modern guideline study
Flag	:	Critical study for SIDS endpoint

31.12.2002 (6)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= at °C
pH value	:	= 10 - 11
concentration	:	100 g/l at 20 °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	
Year	:	

2. Physico-Chemical Data

Id 616-45-5
Date 31.12.2002

GLP	:	no data
Test substance	:	
Remark	:	pH of solution is from: BASF AG, Sicherheitsdatenblatt Pyrrolidon dest. (28.06.1993)
Result	:	Miscible
Test substance	:	CAS No. 616-45-5 2-Pyrrolidone
Reliability	:	(2) valid with restrictions 2 Handbook value
Flag	:	Critical study for SIDS endpoint

06.10.2002 (25)

3. Environmental Fate and Pathways

Id 616-45-5
Date 31.12.2002

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	.000000000012 cm³/(molecule*sec)
Degradation	:	ca. 50 % after 10.8 hour(s)
Deg. product	:	
Method	:	
Year	:	2002
GLP	:	
Test substance	:	

Result :

SMILES : C1CCC(=O)N1
CHEM : 2-Pyrrolidone
MOL FOR: C4 H7 N1 O1
MOL WT : 85.11
- SUMMARY (AOP v1.90): HYDROXYL RADICALS --------
Hydrogen Abstraction = 6.4334 E-12 cm3/molecule-sec
Reaction with N, S and -OH = 5.5000 E-12 cm3/molecule-sec
Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds = 0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings = 0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec

OVERALL OH Rate Constant = 11.9334 E-12 cm3/molecule-sec
HALF-LIFE = 0.896 Days (12-hr day; 1.5E6 OH/cm3)
HALF-LIFE = 10.756 Hrs

Source	:	Toxicology and Regulatory Affairs
Test substance	:	CAS No. 616-45-5 2-Pyrrolidone
Reliability	:	(2) valid with restrictions Calculated by acceptable method
Flag	:	Critical study for SIDS endpoint

08.12.2002 (18)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	> 1 year at 25 °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	
Year	:	2002
GLP	:	no
Test substance	:	

Method : Estimation using HYDROWIN 1.67.
Input was SMILES notation: C1CCC(=O)N1

3. Environmental Fate and Pathways

Id 616-45-5
Date 31.12.2002

Remark : Furthuer supports comes from the "Handbook of Chemical Property Estimation Methods" (2) in which is it is indicated that the mean hydrolytic half-life for a series of amides is in the range of 300 years

(2) J.C. Harris in Lyman W, Reehl, W and Rosenblat, D. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washingotn D.C. 1990, page 7-6
This estimated is supported by the known properties of amides.

For example in the textbook "Organic Chemistry" (1), Vollhardt states that "Amides are the least reactive of the carboxylic derivatives, mainly because of the extra resonance capacity of the nitrogen lone electron pair. As a consequence, their nucleophilic addition-eliminations require relatively harsh conditions. For example, hydrolysis occurs only on prolonged heating in strongly acidic or basic water"

(1) Vollhardt, K. "Organic Chemistry" WH Freeman and Co, New York, 1987, p 815.

Result : HYDROWIN Program (v1.67) Results:
==========
SMILES : C1CCC(=O)N1
CHEM : 2-Pyrrolidone
MOL FOR: C4 H7 N1 O1
MOL WT : 85.11

---- HYDROWIN v1.67 Results ----------

AMIDE: -N-C(=O)-C-
Compound has an amide group; C=O located at SMILES atom #4
Hydrolysis Rate Extremely Slow or t1/2 > 1 Year

Source : Toxicology and Regulatory Affairs
Test substance : 2-Pyrrolidone CAS No. 616-45-5
Reliability : (2) valid with restrictions
Estimated using an acceptable method with comfirmation from both chemical principles and experimental data on surrogate compounds.
Flag : Critical study for SIDS endpoint
30.11.2002 (19)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media : other: all
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other
Year : 2002

3. Environmental Fate and Pathways

Id 616-45-5
Date 31.12.2002

Method : Determined using the Level 3 EQC Model found in EPIWIN 3.05. Actual values were used for measured physicochemical parameters. The degredation times applied using the BIOWIN were validated by experimental data on the test substance and/or surrogate compounds

Result :

Level III Fugacity Model (Full-Output):
==========

Chem Name : 2-Pyrrolidone
Molecular Wt: 85.11
Henry's LC : 1.44e-008 atm-m3/mole (Henrywin program)
Vapor Press : 0.00949 mm Hg (user-entered)
Log Kow : -0.71 (user-entered)
Soil Koc : 0.0799 (calc by model)

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.403	21.5	1000
Water	46.5	360	1000
Soil	53	360	1000
Sedimet	0.0776	1440	0

	Fugacity (atm)	Reaction (kg/hr)	Advect (kg/hr)	Reaction (percent)	Advection (percent)
Air	1.36e-011	153	47.4	5.09	1.58
Water	4.62e-013	1050	547	35.1	18.2
Soil	1.94e-011	1200	0	40	0
Sed	3.85e-013	0.439	0.018	0.0146	0.00061

Persistence Time: 392 hr
Reaction Time: 489 hr
Advection Time: 1.98e+003 hr
Percent Reacted: 80.2
Percent Advected: 19.8

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
Air: 21.51
Water: 360
Soil: 360
Sediment: 1440
Biowin estimate: 2.957 (weeks)

Advection Times (hr):
Air: 100
Water: 1000
Sediment: 50000

Source :

Calculated by Toxicology and Regulatory Affairs, 2002

Test substance :

CAS No. 616-45-5 2-Pyrrolidone

Reliability : (1) valid without restriction
Calculated by an acceptable method using measured physicochemical parameters.

31.12.2002 (18)

3. Environmental Fate and Pathways

Id 616-45-5
Date 31.12.2002

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	other: activated sludge, non-adapted
Contact time	:	
Degradation	:	> 90 (±) % after 9 day(s)
Result	:	
Kinetic of testsubst.	:	1 day(s) = 5 % 5 day(s) = 80 % 7 day(s) = 89 % 9 day(s) = 99 % %
Method	:	Triplicate determinations were made using the test substance at a final concentration of about 500 mg/L and in 2 L of culture containg 100 ml of non-adapted sludge. Elimination was determined by measuring total organic carbon (TOC) at 0 and 3 hours; and at 1, 5, 7, and 9 days after start of the test. The methodology follows the Zahn Wellens test procedure.
Remark	:	Although the conditions do not meet the OECD 301 series, the results clearly demonstrate that non-adapted sludge flora are capable of fully degrading the test material in a short time.
Test substance	:	2-Pyrrolidone, Distilled
Conclusion	:	The test material is considered "inherently biodegradable" showing rapid biodegredation.
Reliability	:	(2) valid with restrictions The raw data for this triplicate determination was available for review; although some details were missing the method is scientifically defensible.
31.12.2002		(7)

Type	:	aerobic
Inoculum	:	
Contact time	:	
Degradation	:	(±) % after
Result	:	readily biodegradable
Deg. product	:	
Method	:	other: estimation
Year	:	
GLP	:	
Method	:	The structure was run through BIOWIN 4.00, as found in EPIWIN 3.05. This software predicts, with excellent accuracy, the ease and relative rate of aerobic biodegredation. Estimates are primarily based on a fragment approach.
Remark	:	This estimate is supported by the high rate of biodegredation observed in the Zahn Wellens procedure (BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung (Pyrrolidon dest., 1977)) and the ready biodegredability of the N-methyl derivitive (NMP, see HSDB) which, based on judgement and BIOWIN modeling, is expected to be slightly more difficult to biodegrade than 2-Pyrrolidone.

3. Environmental Fate and Pathways

Id 616-45-5
Date 31.12.2002

Result :

SMILES : C1CCC(=O)N1
CHEM : 2-Pyrrolidone
MOL FOR: C4 H7 N1 O1
MOL WT : 85.11

BIOWIN v4.00 Results

Linear Model Prediction : Biodegrades Fast
Non-Linear Model Prediction: Biodegrades Fast
Ultimate Biodegradation Timeframe: Weeks
Primary Biodegradation Timeframe: Days
MITI Linear Model Prediction : Biodegrades Fast
MITI Non-Linear Model Prediction: Biodegrades Fast

LINEAR BIODEGRADATION PROBABILITY 0.9172
NON-LINEAR BIODEGRADATION PROBABILITY 0.9889

MITI LINEAR BIODEGRADATION PROBABILITY 0.6448
MITI NON-LINEAR BIODEGRADATION PROBABILITY 0.8408

A Probability Greater Than or Equal to 0.5 indicates --> Readily Degradable
A Probability Less Than 0.5 indicates --> NOT Readily Degradable

SURVEY MODEL - ULTIMATE BIODEGRADATION 2.9569
SURVEY MODEL - PRIMARY BIODEGRADATION 3.9304

Interpretation, Primary & Ultimate:
Result Classification:
5.00 -> hours
4.00 -> days
3.00 -> weeks
2.00 -> months
1.00 -> longer

Test substance :

2-Pyrrolidone CAS No. 616-45-5

Reliability : (2) valid with restrictions
Estimated using an acceptable method.

31.12.2002

Type : aerobic
Inoculum : activated sludge, domestic
Contact time : 28 day(s)
Degradation : = 73 (±) % after 28 day(s)
Result : readily biodegradable
Deg. product :
Method :
Year :
GLP :
Test substance : other TS

Method : Japanese MITI test
Remark :

3. Environmental Fate and Pathways

Id 616-45-5
Date 31.12.2002

Surrogate material

Test substance :

1-Methyl-2-pyrrolidinone CASNO 872-50-4
Surrogate material

Reliability : (2) valid with restrictions
Published study result

Flag : Critical study for SIDS endpoint
31.12.2002 (16)

4. Ecotoxicity

Id 616-45-5
Date 31.12.2002

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 4600 measured/nominal
LC0	:	= 4600 measured/nominal
LC50	:	= 4600 - 10000 measured/nominal
LC100	:	= 1000 measured/nominal
Limit test	:	
Analytical monitoring	:	yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	
GLP	:	yes
Test substance	:	

Method :

METHOD: Followed standard laboratory protocol for OECD 203 (April 1984).
DETAILS OF TEST: Static
DILUTION WATER SOURCE: Municipal water, carbon treated
DILUTION WATER CHEMISTRY: pH 8.0-8.6, total hardness about 2.5 mmol/L, acid capacity about 5.5 mmol/L, TOC not given, TSS not given.
STOCK AND TEST SOLUTION PREPARATION: Test substance added neat to test water 20 minutes before placing fish in aquaria.
VEHICLE/SOLVENT AND CONCENTRATIONS: Dilution water, concentrations 0, 50, 100, 1000, 2150, 4640, 10000 mg/L
STABILITY OF THE TEST CHEMICAL SOLUTIONS: Assured by analytical determination
EXPOSURE VESSEL: All-glass aquaria, 30 x22 x 24 cm, containing 10 L water and filled to a depth of about 17 cm.
REPLICATES, FISH PER REPLICATE: One replicate, 10 fish per replicate
TEMP PHOTOPERIOD FOOD: Test temperature 22-23 °C, photoperiod 16 hours light and 8 hours dark, food withdrawn one day before exposure,
ANALYTICAL CHEMISTRY DETERMINATIONS: TS measured at one and 96 hours.

Result :

Nominal concentrations were: 50, 100, 1000, 2150, 4640 or 10000 mg/L for test.

Analytical concentrations were: 53, 95, 959, 2146, 4580 or 10221 mg/L at one-hour

Analytical concentrations were: 38, 98, 947, 2084, 4600 or 9935 mg/L at 96-hours

pH measurements at one hour were control to high concentration: 8.6, 8.5, 8.4, 8.5, 8.6, 8.6, 8.6; at 96 hours 8.3, 7.0, 9.8, 8.2, 8.2, nd.

Oxygen levels were above 7 mg/L in most instances at 1, 24, 48, 73, or 96 hours.

Temperature remained at 22° throughout the study.

Mortality: There was no mortality except at the high concentration (10,000

mg/L) where the cumulative mortality at 24 hours was 6/10, at 48 hours was 8/10 at 72 and 96 hours was 10/10.

Clinical signs: The only reported effects were for the 10,000 mg/L group at 24 hours where apathy and tumbling were reported in surviving fish.

Test substance :
2-Pyrrolidone CAS No. 616-45-5 Purity 99.7%

Conclusion :
The 96-hour LC50 was between 4,600 and 10,000 mg/L

Reliability : (1) valid without restriction
Guideline study under GLP with no significant problems noted.

Flag : Critical study for SIDS endpoint

31.12.2002 (11)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC0 : = 500 measured/nominal
EC50 : > 500 measured/nominal
Limit Test : no
Method : Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year :
GLP :
Test substance :

Method : Daphnia magna (2-24 hours old) were exposed to the test substance in four replicates of five animals (20/group) at nominal concentrations of 0, 31.25, 62.5, 125, 250, or 500 mg/L for 48 hours. The dilution water was prepared from tapwater by dilution with distilled water to reduce the hardness, addition of sulfuric acid to reduce the alkalinity, filtration to remove particulates and passing the water through activated carbon to remove chlorine. Final dilution water had a total hardness of 2.44 mmol/L, an alkalinity of 0.80 mmol/L (to pH 4.3), a calcium:magnesium ratio (molar) of 4:1, a sodium:potassium (molar) ratio of 10:1 and a pH range of 7.7 to 8.3.

Loading of daphnids was 2 ml/daphnid using 10 ml centrifuge tubes. The temperature was maintained at 293 deg K. Diffuse light was on 16 hours/day at an intensity of 570 microSiemens/cm. The dilution water was bubbled with oil-free air initially to saturate it with oxygen. The test substance dilutions were prepared from a stock at 500mg/l (also the high concentration) by dilution.
Daphnids were examined at 3, 6, 24 and 48 hours after initiation.

Result :

The initial pH did not differ between concentrations and was in the range of 8.11-8.27. The final pH was not concentration dependent and ranged from 7.59 to 8.14. Oxygen concentrations, measure at 0 and 48 hours of the test, were higher at the beginning (9.30-9.42 mg/L) than at the end of the 48 hour exposure period (5.54-8.55) and there was no apparent relationship of DO levels to test-substance concentration.

No daphnids was found immobilized by the treatment and no adverse effects were reported at any concentration.

4. Ecotoxicity

Id 616-45-5
Date 31.12.2002

Test substance :
2-Pyrrolidone CAS No. 616-45-5, distilled, purity > 99.5%

Conclusion :
The NOEC and EC-0 were found to be 500 mg/L
The EC-50 was found to be > 500 mg/L

Reliability : (1) valid without restriction
Guideline study, with good documentation including copies of raw data. Although the test did not use analytical measurements of TX concentration, it is known to be stable in water.

Flag : Critical study for SIDS endpoint
31.12.2002 (8)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 96 hour(s)
Unit : mg/l
EC0 : = 1000 measured/nominal
EC50 : > 1000 measured/nominal
Analytical monitoring : no

Method : Groups of 20 Daphnia magna were exposed to the test substance at either 10, 100, or 1000 mg/L. Groups were made up of four replicates of five daphnids in 300 ml of dilution water containing test substance. Observations were made at least at 24 hours, 96 hours, 7 days, 14 days and 21 days.

Remark :
The stability of the test substance in water was not established. Other information support the test substance being stable in water for at least the initial 48 hour period. Stability at the 3-week time was likely compromised by biodegradation of the test substance.

Result :
No mortality occurred in the first 96 hours of exposure in any group. At the end of the three-week exposure period the number of surviving daphnids was 17/20, 18/20 and 12/20 for the 10, 100 and 1000 mg/L groups, respectively.

Test substance :
2-Pyrrolidone

Conclusion :
The 96-hour EC50 for Daphnia magna is > 1000 mg/L under these conditions.

Reliability : (2) valid with restrictions
Although this study is old and details are limited, the conduct was similar to modern guidelines and the study was conducted according to a scientifically defensible method. The availability of the original data sheets add to the reliability of the work.
31.12.2002 (26)

Type : static
Species : Daphnia pulex (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : = 13.21 calculated
Analytical monitoring : no

Method : Daphnia pulex were cultured in 2-L jars of reconstituted hard water (20OC; pH,7.6-8.0; dissolved oxygen, 60-100% saturation; hardness 160-180 mg/L as CaCO ; alkalinity 110-120 mg/L as CaCO). To minimize leaching,

dissolution and sorption of toxicants from the water only glassware and tubing made from perfluorocarbon plastic was used for culturing and testing. The daphnid food was a mixture of the four algal species plus cerophyl at a ratio of 1:1:1:1:4. The daphnids were fed five times a week with 3 mL of food per liter of culture water.

The 48-h tests were conducted with 10 neonates (<24 h old) in five concentrations of each toxicant and the control. Toxicant concentrations (in 150 mL of reconstituted hard water) were at least 50% of the next concentration. The six test beakers, covered with parafilm, were placed in a constant temperature water bath at 20 deg C with a photoperiod of 16 h light, 8 h dark. Test animals were not fed during the experiment. After 48 h the daphnids were pipetted into a watch glass and examined for immobilization.

Mean effective concentration (EC50) and standard error were calculated from the immobilization data for valid toxicity tests (American Society for Testing and Materials 1980). A mean was taken from three valid tests. To calculate EC10, EC50, and EC90 values, we used a computer modification (Peltier et al. 1985) of Finney's (1952) probit analysis. Statistical comparisons were made on logarithmically transformed EC50's using analysis of variance (ANOVA) and Tukey's HSD test (Steel and Torrie 1960).

(Finney DH (1952) Statistical methods in biological assay. C. Griffin and Co Ltd., London, 661 pp)

(Peltier WH, Weber CI(eds) (1985) Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 3rd ed Environ Monitor Support Lab,US Environ Protect Agency, Cincinnati, Rep no 600/4-85-013)

(Steel RGD, Torrie JH (1960) Principles and Procedures of Statistics, McGraw Hill, New York)

Result :

The results from all studies in ther report are presented in the table below:

Compound	EC50 (mg/L) Mean	SE
DDT (D. magna)	0.0011	0.0001
DDT (17 C)	0.0019	0.0001
Chlordane (D. magna)	0.097	0.005
Nicotine	0.242	0.02
Nicotine (170C)	0.326	0.074
Pentachlorophenol (D. magn)	2.00	0.0
Pentachlorophenol	2.5	0.1
1-methylpyrrolidine	2.08	0.20
Isoxanthopterin	2.97	0.47
2-amino-4,6-dimethylpyridine	9.19	1.85
2-pyrrolidinone	13.21	4.02
2-(2-hydroxyethyl)pyridine	13.82	3.60

Mortality as a function of concentration was not given in the article.

The range of toxicity and the reported SE indicate that studies were conducted in the appropriate concentration range for each test material.

Test substance :

4. Ecotoxicity

Id 616-45-5
Date 31.12.2002

2-Pyrrolidone CAS No. 616-45-5 Purity >= 97%

Reliability : (2) valid with restrictions
High, this is a published study by a National Laboratory in a peer reviewed journal conducted using a scientifically defensible method. Stability data on the test compound are lacking.

31.12.2002 (24)

Type : static
Species : other aquatic mollusc: Planorbella trivolvis
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 112 measured/nominal
EC0 : = 112 measured/nominal
EC50 : > 112 measured/nominal
Limit Test : yes
Analytical monitoring : no

Method : One group of 10 snails was exposed to a solution of 100 microliters/L test substance at a temperature of 18 C for a period of 96 hours. The initial dissolved oxygen level was 9.2 mg/L and the initial pH was 7.7. The final dissolved oxygen level was 2.6 mg/L with a final pH of 7.2. The snails were identified as Helisoma trivolvis, which are currently known as Planorbella trivolvis.

Result :
All snails survived the 96-hour exposure period.

Test substance :
2-Pyrrolidone

Conclusion :
The 96-hour EC50 for Planorbella trivolvis is > 112 mg/L under these conditions.

Reliability : (2) valid with restrictions
31.12.2002 (26)

Type : static
Species : other aquatic worm:
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 112 measured/nominal
EC0 : = 112 measured/nominal
EC50 : > 112 measured/nominal
Limit Test : yes
Analytical monitoring : no
Method :
Year :
GLP : no data
Test substance :

Method : One group of 10 worms was exposed to a solution of 100 microliters/L test substance at a temperature of 18 C for a period of 96 hours. The initial dissolved oxygen level was 9.2 mg/L and the initial pH was 7.7. The final dissolved oxygen level was 2.6 mg/L with a final pH of 7.2. The aquatic worms were identified as Dugesia tigrine, which is a common freshwater platyhelminth.

Test substance :
2-Pyrrolidone

Conclusion :
The 96-hour EC50 for Dugesia tigrine is > 112 mg/L under these

conditions.

Reliability : (2) valid with restrictions
31.12.2002 (1) (26)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC10	:	= 8 calculated
EC50	:	= 84 calculated
Limit test	:	
Analytical monitoring	:	no
Method	:	other: DIN 38412 L9
Year	:	
GLP	:	no
Test substance	:	

Method : Cells were placed in quadruplicate cultures of growth medium according to the method of DIN 38412 L9 containing 0, 25, 50, 100, 250 or 500 mg/L test substance. These concentrations were selected on the basis of a preliminary test at concentrations of 0, 5, 50 or 500 mg/L. Cell counts were determined by counting six replicates from each quadruplicate culture at 0, 24, 48, 72 and 96 hours of incubation. Fluorescence was also determined at these same time-points. pH was measured at the beginning and end of the 90-hour incubation period. The temperature of incubation was a constant 24.8 deg. C.
Statistical Method: Tallerida and Jacob, The Dose-Response Relation in Pharmacology Pages 98-103 pub. Springer Verlag 1979

Remark :

the ECOSAR (v0.99f) program using the neutral orgaincs model predicts a 96-hour EC50 of 4777

Result :

The following results are listed in the order
0, 25, 50, 100, 250 or 500 mg/L:
The beginning and end pH values were
Start: 7.84, 7.87, 7.89, 7.86, 7.89, 7.88
End :7.92, 7.99, 8.04, 8.07, 8.12, 8.13

Mean cells counts (X 1000) were:

t= 0: 34, 38, 32, 34, 33, 35
t=24: 106, 94, 88, 62, 51, 51
t=48: 235, 191, 165, 150, 149, 136
t=72: 618, 514, 405, 239, 311, 230
t=96: 1866, 1408, 1042, 334, 279, 407

The changes in fluorescence did not correlate with the cell growth.

From these data the EC10 and EC50 for growth rate at 96 hours were determined to be 20 and 353 mg/L and the EC10 and EC50 for biomass were determined to be 8 and 84 mg/L.

The 72-hour EC10 and EC50 for biomass were 4 and 253 mg/L

4. Ecotoxicity

Id 616-45-5
Date 31.12.2002

Test substance :
2-Pyrrolidone CAS No. 616-45-5, distilled, purity > 99.5%
Reliability : (1) valid without restriction
Guideline study, with good documentation.
Flag : Critical study for SIDS endpoint
31.12.2002 (9)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : aquatic
Species : Pseudomonas putida (Bacteria)
Exposure period : 17 hour(s)
Unit : mg/l
EC10 : = 9268 calculated
Analytical monitoring : no
Method : other: Bringmann-Kuehn Test
Year : 1988
GLP : no
Test substance :

Method : Bacteria were added to flasks containing salts, dilute growth substrate and test material at 0, 156.25, 312.5, 625, 1250, 2500, 5000, 7500, or 10000 mg/L test material. Flasks were incubated for 17 hours at 297 deg K and bacterial growth was estimated by absorption of light at 436 nm.
Remark : At concentrations below 10,000 mg/L, the test substance appears to have stimulated bacterial growth under these conditions.
Result : Bacterial growth, expressed as percent of control after 17 hours incubation was

TS Conc mg/L	Bacterial growth % of control
0	100
156.25	159
312.5	160
625	162
1250	159
2500	150
5000	151
7500	129
10000	73

Test substance : 2-Pyrrolidone, Distilled
Conclusion : The EC10 was calculated to be 9268 mg/L
Reliability : (2) valid with restrictions
Guideline-type study using a scientifically defensible method.
Documentation good.
08.12.2002 (13)

5. Toxicity

Id 616-45-5
Date 31.12.2002

5.1.1 ACUTE ORAL TOXICITY

Type	:	other: Limit Test
Value	:	> 5000 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	water
Doses	:	5000 mg/kg
Method	:	
Year	:	1979
GLP	:	no data
Test substance	:	
Method	:	Five rats of each sex were given a single oral dose of test material by oral gavage at a limit dose of 5000 mg/kg-bw. The test material was dissolved in distilled water and administered as a 50% wt/vol solution to Sprague-Dawley rats that had been fasted overnight. Male rats weighed approximately 250 grams and females approximately 200 grams at the time of dosing. Animals were observed regularly for mortality and adverse clinical signs and were weighed on days 4, 7 and 13.
Result	:	No animal died during the study. Average body weights of males were 250, 236, 269 and 297 g on days 0,4, 7 and 13, respectively. Average body weights of females were 200, 201, 211 and 216 g on days 0,4, 7 and 13, respectively. No adverse clinical findings were reported.
Test substance	:	2-Pyrrolidone, Pure
Conclusion	:	The acute oral LD50 of the test substance is greater than 5000 mg/kg bodyweight for both male and female rats.
Reliability	:	(2) valid with restrictions Reliability is good as a standard procedure was followed; however, the study lacks details concerning observations and necropsy.
Flag	:	Critical study for SIDS endpoint
30.11.2002		(5)

Type	:	LD50
Value	:	ca. 8000 mg/kg bw
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	water
Doses	:	
Method	:	
Year	:	1961
GLP	:	no
Test substance	:	
Method	:	The study was conducted as part of the "toxicological pre-testing" for this material. The pre-testing consisted of acute oral dosing of rats, inhalation risk-test in rats, i.p. ALD determination in mice, skin and eye irritation. Details of each procedure are not given in the report.
Result	:	In this study, the ALD50 (Approximate Median Lethal Dose) was stated as about 8.0 g/kg at both 24 hours and 8 days. It is presumed that the observation time was 8 days. Clinical signs were given as convulsions,

dyspnea and lying on side; however, it cannot be determined from the report if these signs refer to mice administered TS i.p. or the rats administered TS orally. Likewise, there is no indication of the dose corresponding to these signs or the time of their occurrence.

Test substance : 2-Pyrrolidone, Distilled, solid

21.11.2002 (12)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	other: Inhalation Risk Test
Value	:	
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	6
Vehicle	:	
Doses	:	
Exposure time	:	8 hour(s)
Method	:	other: BASF Inhalation Risk Test
Year	:	1961
GLP	:	no
Test substance	:	

Method : The study was conducted as part of the "toxicological pre-testing" for this material. The pre-testing consisted of acute oral dosing of rats, inhalation risk-test in rats, i.p. ALD determination in mice, skin and eye irritation. Details of each procedure are not given in the report.

Result : Under the conditions of this study no animal died as a result of the exposure to saturated vapor for 8 hours. It is noted in the report that no abnormalities were detected at necropsy; however, the length of the post-exposure observation period is not specified in the report.

Test substance : 2-Pyrrolidone, Distilled, solid

Conclusion : It can be concluded that the 8-hour inhalation LD50 for 2-Pyrrolidone is greater than the air saturation concentration of the test substance in air at 30 deg C. Which is approximately 80 ppm.

Reliability : (2) valid with restrictions
A reliability of 2 is assigned. Although some important details are lacking this study was conducted according to a standard procedure that is scientifically defensible.

21.11.2002 (12)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 2000 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	10
Vehicle	:	
Doses	:	2000
Method	:	OECD Guide-line 402 "Acute dermal Toxicity"
Year	:	1992
GLP	:	yes

5. Toxicity

Id 616-45-5
Date 31.12.2002

Test substance :

Method :

Following a quarantine period of at least one week, five healthy male and five healthy female New Zealand Albino rabbits were randomly assigned to the treatment group. The pretest weight range was 2.3 - 2.6 kg for males and 2.1 - 2.5 kg for females. The animals were housed 1/cage in suspended wire mesh cages. Bedding was placed beneath the cages and changed twice/week. Fresh Purina Rabbit Chow (Diet #5321) was provided daily. Water was available ad libitum. The animal room, reserved exclusively for rabbits on acute tests, was temperature controlled, had a 12 hour dark/light cycle.

The test article was used as received and the dose was based on the sample weight as calculated from the specific gravity. The test article was applied to the prepared dermal site, one time, by syringe type applicator at a dose level of 2.0 g/kg. The test site was covered with a gauze patch, secured with non-irritating tape and gentle pressure was applied to the gauze to aid the distribution of the test article over the area. The torso was wrapped with plastic that was secured with non-irritating tape. At 24-hours after initiation, the patches were removed and residual test article was removed with distilled water.

The animals were observed 1, 2 and 4 hours post dose and once daily for 14 days for toxicity and pharmacological effects. Animals were observed twice daily for 14 days for mortality. The test sites were scored for dermal irritation at 24 hours post dose and on days 7 and 14 using the numerical Draize scale
Body weights were recorded pretest, weekly and at death or termination. All animals were examined for gross pathology. Abnormal tissues were preserved in 10% buffered formalin and saved for possible future microscopic examination.

Result :

All animals survived the 2000 mg/kg dermal application. There were no abnormal systemic signs noted in 9/10 animals. One male exhibited red staining of the nose/mouth area and an apparent cataract in the right eye on day 5, with the ocular abnormality persisting through day 14 but this was considered to result from a slef-inflicted injury unrelated to test material administration. Body weight gains were normal at all weighing periods. Dermal reactions were slight to well-defined on day 1 but were absent on days 7 and 14. Necropsy did not reveal any treatment related changes.

Test substance : 2-Pyrol, no further information
Conclusion : The dermal LD50 was found to be > 2000 mg/kg-bw
Reliability : (1) valid without restriction
Guideline study under GLP with no significant problems noted.
Flag : Critical study for SIDS endpoint
30.11.2002 (23)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.4 REPEATED DOSE TOXICITY

Type : Sub-chronic
Species : rat

5. Toxicity

Id 616-45-5
Date 31.12.2002

Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	drinking water
Exposure period	:	90 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	600, 2400, 7200 or 15000 ppm in drinking water
Control group	:	yes, concurrent vehicle
NOAEL	:	= 2400 ppm
LOAEL	:	= 7200 ppm
Method	:	OECD Guide-line 408 "Subchronic Oral Toxicity - Rodent: 90-day Study"
Year	:	1981
GLP	:	yes
Test substance	:	

Method : 2-Pyrrolidone was administered to groups of 10 male and 10 female Wistar rats at doses of 0; 600; 2,400; 7,200 and 15,000 ppm in the drinking water over a period of 3 months.

Wistar rats (Chbb: THOM (SPF)) were obtained from Dr. Karl Thomae GmbH, Biberach/Riss, FRG. Rats were identified unambiguously by ear tattoo. Animals were individually housed in type DK III stainless steel wire cages Becker & Co., Castrop-Rauxel). Animal rooms were air-conditioned with temperatures in the range 20 - 24°C and relative humidity in the range 30 - 70%. The day/night cycle was 12 hours (light from 06.00 a.m. - 06.00 p.m.).

Test solutions were analysis at the start and end of the study to assure that the concentrations were correct and the 4-day stability was assessed as 97%. The mixtures were prepared at no less than 4-day intervals. Water consumption was determined once/week over a period of 4-days. Animals were weighed weekly and given a thorough physical examination at each weighing. Food consumption was determined weekly. Urine samples were taken on day 85, blood was sampled and analyzed on study day 88, the final bodyweight was recovered on day 91 and necropsies were conducted over days 92 to day 95,

Food consumption, water consumption and body weight were determined each week. The animals' state of health was checked each day. When the animals were weighed they were subjected to an additional comprehensive clinical examination

Clinincal chemistry parameters were: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase - serum-gamma-glutamyltransferase

Blood chemistry parameters were: sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, magnesium.

In addition complete hematology and urinalysis were conducted.

At necropsy, major organs were weighed and sections were fixed for histopathology. All animals were subjected to gross-pathological assessment, followed by histopathological examination using a complete tissue list.

5. Toxicity

Id 616-45-5
Date 31.12.2002

Statistical methods: Means and standard deviations for the variables food consumption, body weight, body weight change, water consumption and test substance intake (except control group) were calculated for the animals of each test group. They were printed out in the summary and individual value tables, with the exception that for test substance intake and body weight change only summary tables were prepared. For the parameters food consumption, water consumption, body weight and body weight change a parametric one-way analysis of variance was done via the F-test (ANOVA). If the resulting p-values were equal to or less than 0.05, a comparison of each dose group with the control group was carried out. These comparisons were performed simultaneously via Dinnett's test for the hypothesis of equal means. If the results of this test were significant, labels (* for,$p < 0.05$, ** for $p < 0.01$) were printed together with the group means in the tables. Both tests were performed two-sided. Statistical analysis of histopathology was conduced with a proprietary computer program.

Remark :

The study was carried out according to following guidelines:

- EC Commission Directive 87/302/EEC of 18 November, 1987; Part B: Methods for the determination of Toxicity; Sub-chronic Oral Toxicity Test; 90-day repeated oral dose using rodent species; Official Journal of the European Communities No. L 133, p. 8-11, 1988

- OECD Guidelines for Testing of Chemicals; Method No. 408: Subchronic Oral Toxicity - Rodent: 90-day study; May 12, 1981

Result :

Substance intake::
Mean test material consumption in mg/kg- day were:
+ males: 33, 184, 529 and 1062 mg/kg
+ females 42, 230, 643 and 1189 mg/kg

No animal died during the study and no adverse clinical signs were noted.

Other effects by dose group:

*** Test group 4 (15,000 ppm; about 1,125 mg/kg body weight)
-decreased food and water consumption in both sexes
- decreased body weight gains, male's BW were 9% lower than controls and female's were 8% lower than controls on day 91
- prolonged prothrombin times in rats of each sex
- decrease in total protein, globulins, triglycerides and creatinine in both sexes
- increased urinary specific gravity in the males - reduced urinary volume in the males
- dark yellow discoloration of urine specimens in the males
- increase in the mean relative kidney weights in males and females

*** Test group 3 (7,200 ppm; about 586 mg/kg body weight)
- slight decrease of food consumption in female animals
- slight decrease of water consumption in both sexes
- slightly decreased body weights in females, 6% less than controls on day 91
- decreased body weight gains of 7% (males) and 16% (females) on day 91
- decrease in creatinine in both sexes
- decrease in total protein in the females

- increased urinary specific gravity in the males - reduced urinary volume in the males
- dark yellow discoloration of urine specimens in the males
- increase in the mean relative kidney weights in males

*** Test group 2 (2,400 ppm; about 207 mg/kg body weight) and
- no substance-related effects

*** Test group 1 (600 ppm; about 37 mg/kg body weight)
- no substance-related effects

Note: A finding of "altered cellular composition of the thymic cortex" was reported in all dosed groups of females. A second 90-day study was conducted at 0, 50 and 15,000 ppm in drinking water using groups of five female rats to investigate the significance of this finding. It this second study the identical finding was present; however, it also occurred in controls. In addition, retrieval and examination of thymus slides from controls animals in other studies were examined and were also found to have the same "pathology". Therefore, this was considered incidental and not compound related.

Test substance :

2-Pyrrolidone CAS No. 616-45-5 Purity 99.7%

Conclusion :

The kidney appears to be a target organ at dose levels of 7,200 ppm (about 586 mg/kg) in the drinking water and above. The NOAEL is 2,400 ppm in drinking water or about 207 mg/kg-bw-day

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
31.12.2002 (10)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Salmonella typhimurium reverse mutation assay
System of testing	:	
Test concentration	:	0, 0.1, 1.0, 5.0, 10, 25, 50, 100 and 150 microliters per plate
Cycotoxic concentr.	:	150 microliters per plate
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1987
GLP	:	yes
Test substance	:	

Method : S. typhimurium strains TA1535, TA1538, TA100, TA1537, TA98 were tested using a plate incorporation technique both with and without metabolic activation. Aroclor 1254 induced rat liver S-9 was used for metabolic activation at a rate of 0.5 ml S-9 per plate when used with the overlay procedure. Test and control materials were incorporated directly into the overlay agar with the bacteria.

Plates were prepared and read in triplicate and the entire assay was repeated a second time (independent repeat). Colonies were counted using an automated Biotran II colony counter except when accurate counts

could not be obtained (e.g. precipitate formation) .

Concentrations of test substance were selected based on a preliminary toxicity assay at 14 concentration levels using two-fold dilutions from a high concentration of 150 microliter per plate (for liquids). As no significant toxicity was observed, 150 microliters per plate was used as the top concentration in the studies.

Concentrations tested were 0, 0.1, 1.0, 5.0, 10, 25, 50, 100 and 150 microliters per plate for all strains in both of the two independent repeats.

The solvent and negative control substance was distilled water. Positive controls were:
Without metabolic activation
Sodium azide at 10 mcg/ plate for strain TA-1535 and TA-100
Quinacrine mustard at 5 mcg/ plate for strain TA-1537
2-Nitrofluorene at 10 mcg/ plate for strains TA-1538 and TA-98

With metabolic activation,
2-Anthramine at 2.5 mcg/ plate for all strains

Statistical Methods

Formal statistical methods were not used to evaluate the data. Evaluations considered if a dose-response was observed and strain-specific evaluation criteria.

For strains TA-1535, TA-1537 and TA-1538, the data set is evaluated as positive if a dose-response is observed over a minimum of three test concentrations and the increase in revertants is equal to or greater than three times the solvent control value at the peak of the dose-response. The solvent control value should be within the normal range for evaluating the results.

For strains TA-98 and TA-l00, the data set is evaluated as positive if a dose-response is observed over a minimum of three test concentrations and the increase in revertants achieves a doubling of the solvent control value at the peak of the dose-response. The solvent control value should be within the normal range for evaluating the results.

Result : In the preliminary study on TA-100, the test material was toxic to the indicator only at 150 microliters per plate as evidenced by the reduced number of revertants on the minimal media plates (about a 50% reduction).

The results of the initial and independent assays conducted on the test material at dose levels ranging from 0.1 to 150 microliters per plate in the absence and presence of metabolic activation did not exhibit increased numbers of his+ revertant colonies.

The positive control treatments in both the nonactivation and S9 activation assays induced large increases in the revertant numbers with all the indicator strains, which demonstrated the effectiveness of the S9 activation system and the ability of the test system to detect known mutagens.

Test substance : 2-Pyrrolidone CAS No. 616-45-5 Purity by GLC 99.9 Area % source BASF

Conclusion : The test material, 2-Pyrrolidone, did not exhibit genetic activity in any of the assays conducted in this evaluation and was not mutagenic to the Salmonella tvphimurium indicator organisms under the test conditions

5. Toxicity

Id 616-45-5
Date 31.12.2002

according to the established evaluation criteria.

Reliability : (1) valid without restriction
Guideline-like study under GLP

Flag : Critical study for SIDS endpoint

28.11.2002 (21)

Type : other: Aneuploidy Induction in Yeast
System of testing : Saccharomyces cerevisiae
Test concentration : 0, 289.6, 321.0, 352.2, 383.3, 414.2, or 445.0 mM
Cycotoxic concentr. : 321 and above
Metabolic activation : without
Result : positive
Method :
Year : 1987
GLP : no data
Test substance :

Method : Diploid strain D61.M of Saccharomyces cerevisiae, developed by F.K. Zimmermann, was used for the detection of aneuploidy and other genetic events. Its genetic constitution and the detailed procedures for its use in detecting aneuploidy have been previously described in detail. In brief: recessive alleles (cyh2, cycloheximide resistance; ade6, white-adenine requirement; leul, leucine requirement) of three genes are arranged on both sides of the centromere on one copy of chromosome VII. Simultaneous expression of all three recessive alleles in the same clone can result either from loss of the homologous chromosome VII carrying the wild-type alleles or from simultaneous multiple events of recombination or mutation, which are expected to be extremely rare.

Ten parallel 5-ml cultures were grown in YEPD medium until they attained a titer of approximately 5-7 x 10exp7 cells/ml. A 0.1-ml aliquot was removed from each culture and plated onto the cycloheximide-YEPD medium to select cultures with low spontaneous rates of cycloheximide resistance. The 5-ml cultures were stored at 4°C until use. A culture that was determined to have a low spontaneous frequency of cycloheximide resistance (typically < 1 x 10exp6) was diluted 1:10 into fresh YEPD medium and incubated at 28C for 4 hr to bring the cells into exponential growth phase before addition of the test chemical.
The exponential phase culture was adjusted to 5 x 10exp6 cells/ml in YEPD medium. Treatments were carried out in 2-nil aliquots in glass test tubes by adding microliter quantities of the test chemical either directly or from a stock solution of the chemical in water prepared just before use. The concentration of the stock solutions was dictated by the level of toxicity, which had been determined in preliminary experiments. The growing yeast cells were treated in a shaker water bath at 28°C for 4 hr; then the cultures were refrigerated at 4°C in a water bath for 16 hr. The cold holding period was followed by a second 4-hr incubation at 28°C before the cultures were diluted and plated on the appropriate media. (The interruption of growth by cold temperature storage greatly enhances the induction of aneuploidy by a number of solvent chemicals). When necessary, cultures were diluted to approximately 1-2 x 10exp7 cells/ml, and 0.1-ml aliquots were plated directly onto the selective cycloheximideYEPD medium to determine the resistant population. Appropriate dilutions were plated onto YLPD medium to determine the surviving population. Plates were incubated for 5-7 days, and colonies were enumerated. On selective cycloheximide-YEPD medium the resistant colonies were either red or white. The red colonies resulted from the occurrence of genetic events such as gene conversion or mutation

affecting the CYH2 locus only and not from chromosome malsegregation. The cycloheximide-resistant white colonies are presumably due to chromosome loss because the recessive cyh2 and the recessive ade6 alleles are being simultaneously expressed. To confirm that the white resistant colonies are really monosomic for chromosome VII, each colony to be tested was streaked onto YEPD master plates, which were incubated overnight at 28C, and then replicas were plated onto both a synthetic complete medium and onto the same medium lacking leucine. White (ade6) and cycloheximide-resistant (cyh2) colonies must also require leucine (leu1) to be considered monosomic.

Remark : In a subsequent paper, these same authors found no aneuploidy potentiation of 2-Pyrrolidinone with nocodazole. They discussed the potential mechanism of solvent-induced aneuploidy in terms of the fact that microtubles dissociate in the cold to their tubulin subunits and polymerize again as the temperature is raised. The solvents were speculated to inhibit or accelerate the rate of repolymerization (Mayer and Goin, Mut Rech. 201:413-421, 1988).
Several factors indicate that this result is not relevant to hazard assessment to man.

Solvent-induced aneuploidy appears to be a special case.

Solvent-induced aneuploidy is enhanced by cold incubation, which was part of the protocol in this investigation.

The concentration range where effects are reported is narrow range and coincides with toxicity.

The concentrations where effects are reported are extremely high and impossible to achieve under normal industrial conditions in man.

Common non-genotoxic solvents such as acetone are known to induce this effect under the special conditions employed in this study.

Result : Positive results on the induction of aneuploidy by 1-methyl-2-pyrrolidirone and 2-pyrrolidinone were recorded as the number of cycloheximide-resistant white colonies observed and the fraction of these colonies that were Leu-. Aneuploidy frequencies were calculated by using these numbers as the numerator and the population screened as the denominator. In cases in which only a few white colonies were found, all were tested for their leucine requirement. When many white colonies were observed, all were counted, and a representative number (usually 25) was tested. The number of red cycloheximide-resistant colonies was determined and was found not to increase with test material concentration. As red-resistant colonies arise as a result of other genetic events, they served as a control showing that other genetic effects such as mutation or recombination were not induced by the test chemical.

The frequency of aneuploidy increased with the dosage of each test chemical. 1-Methyl-2-pyrrolidinone was active between 150 and 230 mM, while 2-pyrrolidinone was active between 350 and 450 mM, and appeared to be slightly less toxic in comparable ranges. As there was no increase with concentration for either chemical in the frequency of the red cycloheximide-resistant colonies. Therefore, aneuploidy rather than other nuclear genetic effects were being induced by these chemicals.

Data are shown in the table.

Test substance : 2-Pyrrolidone CAS No. 616-45-5 from Aldrich Chemical Co

5. Toxicity

Id 616-45-5
Date 31.12.2002

Attached document : Y-table-HP600.bmp

Test M Conc (mM)	Percent Survival	Pop Screened X 10^6	Total White Colonies	Aneuploidy Frequency x10^6 CFU
0	100	4.73	10	1.27
289.6	98	5.20	42	6.79
321.0	61	4.35	48	9.71
352.2	42	4.43	65	10.56
383.3	23	3.28	98	17.93
414.2	8	1.75	120	21.94
445.0	7	1.50	120	19.20

Reliability : (2) valid with restrictions
The method was well described and sufficient details and data were presented to indicate that this study has good reliability.

28.11.2002 (22)

Type : Cytogenetic assay
System of testing :
Test concentration :
Cycotoxic concentr. : High doses minimally cytotoxic.
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 473
Year : 1987
GLP : yes
Test substance :

Method : 2-Pyrrolidone was tested for its ability to induce chromosomal aberrations in human lymphocytes following in vitro exposure in the presence and absence of a metabolizing system.
Based on a pretest to determine the highest experimental dose and in consideration of the cytotoxicity actually found in the present cytogenetic investigations, 3500 mcg/ml, 2500 mcg/ml and 1250 mcg/ml culture medium in the experiment without S-9 mix. or 6000 mcg/ml, 5000 mcg/ml and 2500 mcg/ml culture medium in the experiment with metabolic activation, were selected. This selection was based on the quality of the metaphases and not on the mitotic index because the test substance concentrations causing reduction in the mitotic index are at dose levels that severely affect chromosomes; thus, no longer allowing evaluation.
Duplicate cultures were used for all experimental points. The solvent was distilled water.
Negative controls (untreated and solvent) and positive controls both without S-9 mix (0.2 mcg mitomycin C/ml culture medium) and with metabolic activation (6 mcg cyclophosphamide/ml culture medium) were also tested.

Heparinized human venous blood was added to the culture medium (chromosome medium 1A with PHA). After mitogen stimulation of the lymphocytes using PHA and incubation at 37°C for 48 hours. The cultures were treated with test substance without S-9 mix for 24 hours; in the experiment with S-9 mix (from Aroclor-induced rats) test substance treatment lasted 2 hours followed by a reincubation for 22 hours using

fresh culture medium without test substance. About 2 - 3 hours prior to harvesting the cells, Colcemid was added to arrest cells in a metaphase-like stage of mitosis (C-metaphase). After preparation of the lymphocyte chromosomes and staining with Giemsa, 100 metaphases of each culture in the case of the test substance, untreated control and solvent control, or 50 cells of each culture in the case of positive controls, were analyzed for chromosomal aberrations.

Statistical Procedure:

The Fisher exact test was applied to determine significant differences between the relative frequencies of a characteristic of two groups, and it was used to answer the questions of whether there are significant differences between control groups (untreated controls and solvent controls) and dose groups with regard to the rate of structural aberrant metaphases.

Result : ** Assay without metabolic activation:::
Untreated controls
10 (5.0%) aberrant cells including gaps and 2 (1,0%) aberrant cells excluding gaps were found

Solvent controls:
12 (6.0%) aberrant metaphases including gaps and 5 (2.5%)aberrant metaphases excluding gaps were found

3500 mcg/ml:
8 (4.0%) chromosomally damaged cells including gaps and 2 (1.0%) aberrant cells excluding gaps were detected.

2500 mcg/L:
14 (7.0%) aberrant metaphases including gaps and 6 (3.0%) chromosomally damaged cells excluding gaps were observed.

1250 mcg/ml:
17 (8.5%) aberrant cells including gaps and 2 (1.0%) aberrant metaphases excluding gaps were found.

0.2 mcg mitomycin C/ml:
With 44 (44%) aberrant cells including gaps and 37 (37%) aberrant mitosis excluding gaps including 2 multiple aberrant metaphases and 5 cells with exchanges, the positive control substance led to the expected increase in the number of chromosomally damaged cells.

No differences regarding aneuploidies (hyperploid metaphases) and polyploidies between the various dose groups and the negative controls were observed.

Assay with metabolic activation:::

Untreated control:
4 (2.0%) aberrant mitosis including gaps only were found.

Solvent contro1:
15 (7.5%) aberrant metaphases including gaps and 4 (2.0%) chromosomally damaged cells excluding gaps were found.

6000 mcg/ml:

17 (8.5%) chromosomally damaged cells including gaps and 2 (1.0%) aberrant cells excluding gaps were observed.

5000 mcg/ml:
16 (8.0%) chromosomally damaged cells including gaps and 1 (0.5%) aberrant cells excluding gaps were observed.

2500 mcg/ml:
13 (6.5%) chromosomally damaged cells including gaps and 1 (0.5%) aberrant cells excluding gaps were observed.

6 mcg cyclophosphamide/ml:
27 (27%) chromosomally damaged cells including gaps and 20 (20%) aberrant cells excluding gaps were observed, which was the expected increase for positive controls.

No differences regarding aneuploidies (hyperploid metaphases) and polyploidies between the various dose groups and the negative controls were observed.

Test substance : 2-Pyrrolidone CAS No. 616-45-5 Purity 99.9%

Conclusion : According to the results of the present study, the test substance 2-pyrrolidone did not lead to any increase in the number of aberrant metaphases including and excluding gaps when compared to the solvent controls either without S-9 mix or after adding a metabolizing system. 2-Pyrrolidone is evaluated not to be a chromosome-damaging (clastogenic) agent under in vitro conditions using human lymphocytes, under these experimental conditions.

Reliability : (1) valid without restriction
Guideline study under GLP with no significant problems noted.

Flag : Critical study for SIDS endpoint
29.11.2002 (2)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : NMRI
Route of admin. : i.p.
Exposure period : 16, 24 and 48 hours
Doses : 2000, 1000, and 500 mg/kg-bw
Result : negative
Method : OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year : 1993
GLP : yes
Test substance :

Method :

Male and female animals (NMRI mice, Charles River GmbH, WIGA) were assigned to the test groups using a randomization plan prepared with an appropriate computer program. Animals were housed in Makrolon cages, in groups of 5 according to sex in fully air-conditioned rooms with a range of 20 - 24°C for temperature and a range of 30 - 70% for relative humidity. Before treatment, animals were transferred to Makrolon cages and housed individually under the same conditions until the end of the test. The day/night rhythm was 12 hours (light from 6.00 - 18.00 hours).

Standardized pelleted feed (Kliba Haltungsdidt, Klingentalmühle AG) and drinking water from bottles were available ad libitum.

Doses selected were 2000, 1000 and 500 mg/kg-bw and were selected on the basis of a preliminary toxicity study. In this study, the highest recommended dose of 2000 mg/kg was administered and survived by all animals but led to signs of toxicity such as irregular respiration, piloerection, abdominal position, apathy and squatting posture; the general state of the animals was poor.

Five Male and female animals per sacrifice interval and dose group were given test substance dissolved in distilled water 2000 mg/kg, 1000 mg/kg and 500 mg/kg body weight. Treatment consisted of a single intraperitoneal administration with a volume of 10 ml/kg body weight. As a positive control, 20 mg of cyclophosphamide/kg body weight or 0.15 mg of vincristine/kg body weight, both dissolved in distilled water, were administered to groups (five animals total, either 2 or 3 of each sex) of male and female animals once intraperitoneally each in a volume of 10 ml/kg body weight. All test substance formulations were prepared immediately before administration.

Sacrifice intervals per dose-group were:

2000 mg/kg;	16, 24 and 48 hours
1000 mg/kg;	24 hours
500 mg/kg	24 hours
Controls	24 hours

Preparation of bone marrow: After cutting off the epiphyses, the bone marrow was flushed out of the diaphysis into a centrifuge tube using a cannula filled with fetal calf serum which was at 37°C (about 2 ml/femur). The suspension was mixed thoroughly with a pipette, centrifuged at 1500 rpm for 5 minutes, the supernatant removed the cells were resuspended. One drop of this suspension was dropped onto clean microscopic slides. Smears were prepared using slides with ground edges, the preparations were dried in the air and subsequently stained in eosin and methylene blue solution for 5 minutes, rinsed, placed in fresh distilled water for 2 or 3 minutes and finally stained in Giemsa solution for 12 minutes. After being rinsed twice and clarified with xylene, the preparations were embedded in Corbit-Balsam. Slides were coded before microscopic analysis.

Evlauations: In general, 1000 polychromatic erythrocytes from each male and female animal of every test group was evaluated and investigated for micronuclei. The normochromatic erythrocytes which occur were also scored. The following parameters were recorded:
Number of polychromatic erythrocytes
Number of polychromatic erythrocytes containing micronuclei
Number of normochromatic erythrocytes
Number of normochromatic erythrocytes containing micronuclei
Ratio of polychromatic to normochromatic erythrocytes
Number of small micronuclei (d < D/4) and of large micronuclei (d >_ D/4)

No statistical methods were employed in data analysis.

Result :

Clinical examinations: The single intraperitoneal administration of the solvent in a volume of 10 ml/kg body weight was tolerated by all animals without any signs or symptoms. A dose of 2000 mg/kg body weight of test substance, led to irregular respiration, piloerection, abdominal position and

apathy about 30 minutes after administration; the general state of some animals was poor. After treatment of the animals with 1000 or 500 mg/kg, only irregular respiration and piloerection were observed after about 30 minutes. After about 1 - 2 hours clinical signs were no longer observed. Neither the single administration of the positive control substance cyclophosphamide in a dose of 20 mg/kg-bw nor that of vincristine at 0.15 mg/kg-bw caused any evident signs of toxicity.

Micronulei: Mean polychromatic erythrocytes containing micronuclei were:

Negative control (24 hrs)	1.5%
2000 mg/kg (16 hrs)	1.2%
2000 mg/kg (24 hrs)	1.7%
2000 mg/kg (48 hrs)	1.6%
1000 mg/kg (24 hrs)	2.4%
2000 mg/kg (16 hrs)	1.2%
Cyclophosphamide (24 hrs)	13.6%
Vincristine (24 hrs)	83.2%

Administration of test substance did not lead to any increase in the rate of micronuclei. The number of normochromatic or polychromatic erythrocytes containing small micronuclei (d < D/4) or large micronuclei (d > D/4) did not deviate from the solvent control value at anv sacrifice interval. No inhibition of erythropoiesis induced by the treatment of mice with Pyrrolidon-2 was detected; the ratio of polychromatic to normochromatic erythrocytes was always in the same range as that of the control values in all dose groups.

The number of normochromatic erythrocytes containing micronuclei did not differ to any appreciable extent in the negative control or in the various dose groups at any of the sacrifice intervals.

Test substance : 2-Pyrrolidone CAS No. 616-45-5 Purity > 99.5%
Conclusion :

The number of normochromatic erythrocytes containing micronuclei did not differ to any appreciable extent in the negative control or in the various dose groups at any of the sacrifice intervals.

Reliability : (1) valid without restriction
Guideline study under GLP with no significant problems noted.

Flag : Critical study for SIDS endpoint
29.11.2002 (4)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : days 6-15 of gestation
Frequency of treatm. : Daily
Duration of test :

5. Toxicity

Id 616-45-5
Date 31.12.2002

Doses	:	190, 600, 1900
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 190 mg/kg bw
NOAEL teratogen.	:	= 600 mg/kg bw
Result	:	Not Specific Developmental Toxin
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	
GLP	:	yes
Test substance	:	

Method :

Groups of 25 pregnant rats were exposed to the test substance by oral gavage using distilled water as vehicle at dose levels of 0, 190, 600 or 1900 mg/kg-bw. On day 20 of gestation, each female was killed and given a gross pathological examination. The gravid uterus was weighed, its contents were examined and all the fetuses were weighed and examined externally. Of these fetuses, approximately half were given a fresh internal examination, their heads removed and examined by the technique of Wilson. The remaining fetuses were eviscerated. All fetuses were stained with Alizarin Red S and their skeletons examined.

Female Sprague-Dawley rats [Crl:CD (SD) BR] were obtained from Charles River Breeding Laboratories, Kingston, New York. After arrival, animals were examined by a veterinary aide; any animals found in poor condition were rejected from the study. After an acclimation period of 14 days, each female was placed in a cage with a proven male breeder of the same strain and source. On the day of mating (Day 0 of gestation), the females were 80-93 days of age and weighed between 231 and 320 g. Pregnancy was assumed when there was positive identification of spermatozoa in the daily vaginal lavage and this was termed day 0 of gestation. Animals were individually housed except during mating.

MATERNAL IN-LIFE DATA: Animals were checked twice daily for mortality and clinical signs. Pregnant females were examined prior to and following dosing for reactions to treatment, indications of poor health and abnormal behavior from day 6 to day 15 of gestation. Animals were weighed once each week during the acclimatization period and on days 0, 6, 9, 12, 15, 18 and 20 of gestation. Food intake was assessed for all animals on days 0 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18 and 18 to 20 of gestation. On day 20 of gestation, female rats were killed by carbon dioxide asphyxiation followed by exsanguination from the abdominal aorta, each was given a complete gross pathological examination.

MATERNAL EXAMINATION: The reproductive tract of each female was dissected out, the ovaries removed and the corpora lutea counted. The uterus was weighed. The uterine contents were examined and the number and position of live fetuses, dead fetuses, early (endometrial gland with or without some placental tissue), middle (discernible placental and fetal tissue present) and late (fetal structure apparent) resorptions were recorded. The fetuses were then removed from the uterus for examination. The uterus of any animal judged to be nonpregnant was stained with 10% aqueous (v/v) ammonium sulphide solution and was then examined for implantation sites.

FETAL EXAMINATION: Each fetus was weighed, given a detailed external examination with external sex being recorded and then killed. A detailed internal examination using a dissecting microscope was performed on

approximately one half of the fetuses, selected randomly from each litter, which were then eviscerated. The heads of these fetuses were removed and placed in Bouin's fluid for examination by the technique of Wilson. The remaining fetuses in each litter were eviscerated; these and the bodies of those fetuses examined internally were placed in 85% ethanol/15% methanol for subsequent staining with Alizarin Red S using a modified Dawson technique for skeletal examination.
Abnormalities were classified as major malformations, minor visceral or skeletal anomalies or common skeletal variants.

STATISTICAL METHODS: The group mean body weights and body weight gains of animals with live fetuses were calculated. The group mean corrected body weights for day 20 of gestation (body weight on day 20 minus gravid uterus weight) and the corrected body weight gains from day 6 to 20 (corrected body weight day 20 minus body weight day 6) were calculated (Data for non-pregnant animals were not included). These parameters were analyzed using one-way analysis of variance, and where the F value was found to be of significance ($P < 0.05$), intergroup differences between control and treated groups were examined using Dunnett's "t" test.

The group mean live litter size, corpora lutea count, number of implants and number of resorptions were calculated. The individual and group litter mean for the sex ratio and pre- and post-implantation losses were calculated. Statistical analyses were performed using the Kruskal-Wallis test and where the "H" value was significant ($P <0.05$) the Mann-Whitney "U" test was used to analyze for differences between control and test groups.

The litter mean fetal weights and group mean fetal weights were calculated and statistical analysis was performed using an analysis of variance (one-way classification) and Dunnett's "t" test.

The incidences of major malformations and minor anomalies were reported as the number of litters with abnormalities in each group and the number of fetuses affected. Statistical analyses comparing the number of litters (containing major malformations) in each test group with the control values were performed using either the chi-square test (with Yate's correction factor) or Fisher's exact probability test. The incidence of minor anomalies was analyzed in the same manner. The incidence of common skeletal variants was reported as the litter mean percentage of fetuses affected. Statistical analyses were performed by comparing the litter mean percentage incidences of each test group with the control group using the Kruskal-Wallis and Mann-Whitney "U" tests.

Result :

No animals died during the study and no treatment-related clinical signs were reported.
BODY WEIGHT: Between day 6 and day 9 of gestation, the 1,900 mg/kg-day group lost weight while the body weight gains were significantly reduced in the 600-mg/kg-day group. There were significantly reduced body weight gains over the day 9 to 12 interval in the 1,900-mg/kg-day group. These reduced body weight gains resulted in significantly reduced body weights from day 9 to 20 of gestation in both the 600 and 1,900 mg/kg-day groups. The corrected body weights were significantly decreased in the 600 and 1,900 mg/kg-day groups and the corrected body weight gain was decreased significantly in the 1,900-mg/kg-day group.
FOOD CONSUMPTION: (Table 5, Appendix 3)

Over days 6 to 9 and 9 to 12 of gestation, food consumption in both the 600 and 1,900-mg/kg-day groups was significantly reduced. Food consumption continued to be significantly reduced over days 12 to 15 of gestation in the 1,900-mg/kg-day group only.
GROSS PATHOLOGICAL FINDINGS: (Table 1, Appendix 6)
Gross pathological examinations revealed no abnormalities related to treatment other than a few incidental findings among mid and low-dose animals on the study.

UTERINE FINDINGS: (Tables 1 and 8, Appendix 7)
The pregnancy rate was at least 88.0% in all groups. Ammonium sulphide staining revealed no other pregnancies.
Gravid uterus weights were significantly reduced in the high-dose group. There were no significant differences between control and treated groups for the following ovarian and uterine parameters: total corpora lutea, total implantation sites, numbers of male and female fetuses, sex, ratio, number of live fetuses, number of dead fetuses, early, middle and late resorptions, total resorptions and pre- and post-implantation losses.

FETAL FINDINGS:
FETAL WEIGHTS were significantly reduced for males, females and totals only in the high-dose group.
MAJOR MALFORMATIONS,In the high-dose group there was a significant increase in the incidence of litters and fetuses with major malformations with 5 fetuses affected. All had acaudia or microcaudia and anal atresia. In addition, one of these fetuses had absence of some thoracic and all lumbar, sacral and caudal vertebrae and absence of 9 pairs of ribs. The incidence of major malformations in the mid and low-dose groups was not different from controls.
MINOR VISCERAL ANOMALIES: There was no effect upon the overall incidence of litters with minor visceral anomalies, but the incidence of fetuses affected was significantly increased in the high-dose group.
MINOR SKELETAL ANOMALIES: The overall incidence of fetuses with minor skeletal anomalies was significantly increased at the high dose. This increase was primarily the result of significantly increased incidences of several findings which included reduced ossification of frontal bones, irregular ossification of supraoccipital bones, reduced number of pre-sacral vertebrae and ossification centers on the seventh cervical vertebra. In the mid and low-dose groups, statistically significant differences in the incidences of reduced ossification of the interparietal bone, ossification centers on the first lumbar vertebra, reduced ossification of the pubic bones, reduced ossification of the ischial bones or absent ribs were attributed to intergroup variation.

COMMON SKELETAL VARIANTS:The percentage of fetuses with thoracic centrum variants was significantly decreased in the 1900 mg/kg-day group. There was a statistically significant reduction in the percentage of fetuses with sternebral (5 or xiphisternum) variants in the 190-mg/kg-day group that was attributed to intergroup variation.

The accompanying table presents most of the fetal results in tabular form.

5. Toxicity

Id 616-45-5
Date 31.12.2002

Test substance : 2-Pyrrolidone CAS No. 616-45-5, Purity 99.6%

Attached document : Tab-Dev-01.bmp

Dose(mg/kg)	0	190	600	1900
Dams Pregnant	22	25	23	24
Corpora lutea:	17.5	18.3	17.4	17.5
Implantations:	16.3	16.4	16.4	15.5
Postimplantation Loss:	0.7	0.8	1.0	0.8
Live Fetuses/Litter	15.5	15.5	15.4	14.8
Total # Dead Fetuses	0	0	0	0
Total # Live Fetuses:	341	388	355	354
Mean Fetal Weight (g):	3.45	3.54	3.40	3.12
Sex Ratio (male):	0.43	0.46	0.46	0.51
Major Malformations	0	1	1	5*
Litters with Maj Malf	0	1	1	5*
Minor Visceral Malf.	1	2	1	7
Litters with MVM	1	2	1	5
Minor Skeletal Anoml	82	98	60	140**
Litters with MSA	19	23	19	23

* Statistically Significant

Conclusion :

Treatment of pregnant rats with 2-pyrrolidone, by gavage, at dosages of up to 1,900 mg/kg-day, throughout major organogenesis, resulted in significant maternal toxicity at the 600 and 1,900 mg/kg-day levels, as evidenced by decreased body weights and food consumption. At the 1,900 mg/kg-day level there were increased incidences of major malformations, minor visceral and skeletal anomalies and decreased fetal weights. No effect upon postimplantation loss was observed.
Therefore, 2-pyrrolidone at a dose of 1,900 mg/kg-day was considered embryo- and fetotoxic but not embryolethal. No effect upon embryonic development was seen at the 600 mg/kg/day level where a significant level of maternal toxicity occurred. The 190 mg/kg/day group was considered the no effect level for maternal toxicity. Based upon these data, the A/D (adult/developmental) ratio was calculated to be <1, indicating 2-pyrrolidone did not show selective toxicity to the rat fetus.

Reliability : (1) valid without restriction
Modern Guideline study under GLP

Flag : Critical study for SIDS endpoint
31.12.2002 (14)

Species : rat
Sex :
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : days 6-15 of gestation
Frequency of treatm. : daily
Duration of test : 10 days
Doses : 1700 microliters/kg-bw
Control group : yes, concurrent no treatment
Result : Not teratogenic in the rat by oral gavage
Method : other: FDA 1966

5. Toxicity

Id 616-45-5
Date 31.12.2002

Year	:	1971
GLP	:	no
Test substance	:	
Method	:	Test substance was administered in distilled water to 25 presumed-pregnant dams on days 6-15 of gestation. Dosing solution was prepared fresh daily. Controls (26 dams) were untreated. Animals were checked daily for adverse clinical signs and mortality. Animals were weighed three times a week during the dosing period. The dose of the test substance was based on the weight of the rat on day 0. The concentration of the solutions was adjusted in such a way that the amount of test substance to be administered for 100 g body weight was contained in a volume of 0.5 ml. On the 20th day of post coitum all the animals were sacrificed, the uteri were removed, the implantation and resorption sites were recorded, the number of live and deed fetuses, their body length, their weight and sex, and the weight of the placentas were determined. The fetuses were examined macroscopically for any malformations. A third of the fetuses of each dam were fixed in Bouin's solution and transversal sections were prepared and assessed according to Wilson's method (Wilson, Warkany: Teratology, Principles und Techniques, 1965). For the assessment of the skeletal system, the remaining fetuses were fixed in 96% strength alcohol, clarified with potassium hydroxide solution and stained with Alizarin red-S using a modified Dawson method. The uteri of the apparently nonpregnant animals or the empty uterine horns in the case of single-horn pregnancy were stained in 10% strength ammonium sulfide solution and then assessed again in order to determine early resorptions.
Remark	:	The dose level was 1700 microliters/kg-bw. Based on the specific gravity of 1.103, this is approximately 1875 mg/kg-bw. Without the maternal body-weight gain data the maternal toxicity cannot be adequately assessed. This dose was approximately the same a as that used in the three-dose level 1990 developmental toxicity study and the results are similar in that there was not a major teratogenic effect.
Result	:	All the pregnant rats tolerated the 10 oral administrations of test material without visible signs of toxicity. One dam died on the 17th day post coitum. The animal proved to be not pregnant. No substance-induced changes could be observed macroscopically. The mean number of implantations and the percentage of resorptions did not differ between the test and control groups. Maternal weights, although recorded, were not included the report. MACROSCOPIC FETAL EFFECTS: The mean weight and length of the fetuses in the test group did not differ from the values in the control group. The mean weights of the placentas in the test group and untreated control group were also comparable. The percentage of malformed live fetuses was 2.8 in both groups; similarly, the percentage of runts was the same in the test and control groups. SKELETAL ASSESSMANT: In treated animals, one fetus (dam No. 6) had a bipartite 12th thoracic vertebral centrum. One fetus (dam No. 10) was observed to have anasarca and two other fetuses of this dam had a cleavage of the eleventh thoracic vertebral centrum. Dam No. 22 had one malformed fetus. The tail of this fetus was missing and atresia was also reported. One fetus of dam No. 24 had a bipartite eleventh thoracic vertebral centrum. In Untreated animals: One fetus (dam No. 30) had a bipartite eleventh

5. Toxicity

Id 616-45-5
Date 31.12.2002

thoracic vertebral centrum. One fetus (dam No. 33) had a bipartite twelfth thoracic vertebral centrum. One fetus of each of dams Nom. 34 and 35 had a bipartite eleventh thoracic vertebral centrum. The presphenoid was missing in one fetus of dam No. 44. One fetus of dam No. 47 had a bipartite 12th thoracic vertebral centrum.

TRANSVERSE SECTIONS: No malformations were found in the fetuses of test or control animals.

Test substance : 2-Pyrrolidone CAS No. 616-45-5

Conclusion : The pregnant dams tolerated the 10 oral administrations of test material without any visible symptoms of toxicity or any macroscopically evident pathological changes. The malformations or anomalies found in the fetuses of the test group corresponded in type and number to those of the controls and historical controls. The test material does not have teratogenic effects in Sprague-Dawley rats.

Reliability : (2) valid with restrictions
A reliability of 2 is assigned. Although some important details are lacking this study was conducted according to a standard procedure that is scientifically defensible. It has value as a supporting study.

08.12.2002 (3)

9. References

(1) All flatworms survived the 96-hour exposure period.

(2) BASF AG, Abt. Toxikologie, unpublished study report (86/286), 26.11.1987

(3) BASF AG, Abt. Toxikologie, unveroeffentlichte Untersuchung (XIX/421), 04.08.1971

(4) BASF AG, Abteilung Toxikologie; unpublished report. Cytogenetic Study In Vivo of Pyrrolidon-2 in Mice, Micronucleus test. (92/1491), 28.06.93

(5) BASF AG, Abteilung Toxikologie; unveroeffentliche Untersuchungen (79/409), 09.04.1981

(6) BASF AG, Analytisches Labor; Unpublished Stiudy (J.Nr.129300/04 vom 14.06.88)

(7) BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung (Pyrrolidon dest., 1977)

(8) BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0701/88)

(9) BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0701/88, Fa.Noack)

(10) BASF AG, Report of the Subchronic oral toxicity with 2-Pyrrolidone in Wistar rats, 3-month drinking water, Project No. 52S0014/92038 June 4, 1998

(11) BASF AG: Abt. Toxikologie, unpublished report, (92/14), 01.08.1995

(12) BASF AG: Abt. Toxikologie, unveroeffentlichte Untersuchung,(XI/407), 07.11.1961

(13) BASF Labor Okologie, unpublished study, 28.06.88

(14) Bio-Research Laboratories Inc, An Oral Teratoloty Study of 2-Pyrrolidone in the Rat. Project # 83880, Dec. 19, 1990 Sponsored by GAF Chemicals and BASF AG

(15) Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996. 1378

(16) Chem Inspect Test Inst; Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan; Published by Japan Chemical Industry Ecology-Toxicology & Information Center. ISBN 4-89074-101-1 p. 5-5 (1992)

(17) Daubert, T.E. and Danner, R.P. Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. Design Institute For Physical Property Data, American Institute Of Chemical Engineers. Hemisphere Pub. Corp., New York, NY., 5 Vol, 1997

(18) EPIWIN 3.05 caluclation SRC Syracuse NY

(19) Estimated using HYDROWIN 1.67 as found in EPIWIN 3.05, SRC Syracuse NY

(20) Flick, E.W. (ed.). Industrial Solvents Handbook 4 th ed. Noyes Data Corporation., Park Ridge, NJ., 1991. 918, as cited in Hazardous Substance Data Base, NLM, Revison of 8-6-2002

(21) Jagannath, D.R., Mutagenicity Test on 2-Pyrrolidone in the Ames Salmonella/Microsome Reverse Mutation Assay, Final Report, Hazleton Labs, GAF Sponsor April 24, 1987.

9. References

(22) Mayer, V.W. Goin, C. J. and Taylor-Mayer, R. E. Aneuploidy Induction in Saccharomyces cerevisiae by Two Solvent Compounds, 1-Methyl-2-pyrrolidinone and 2-Pyrrolidinone. Environmental and Molecular Mutagenesis 11:31-40, 1988

(23) MB Research Laboratories Inc project number MB-92-1432 Sponsored by International Specialty Products, 4/29/1992.

(24) Perry, C.M., Smith,S.B. Toxicity of Six Heterocyclic Nitrogen Compounds to Daphnia pulex. Bull. Environ. Contam. Toxicol.41, 604-608, (1988)

(25) Riddick, J.A.; Bunger, W.B.; and Sakano, T.K. Organic Solvents: Physical Properties And Methods Of Purification. Techniques Of Chemistry. 4th Ed. New York, NY: Wiley-Interscience. 2: Pp.1325, 1986 (as cited in CIS 4-2002)

(26) Submission to U.S. EPA: Raw data for ecotoxicity information on 2-Pyrrolidinone (CAS Reg No 616-45-5), with cover letter dated 01/29/86 Source: EPA/OTS; Doc #FYI-OTS-0794-1152 Submitted by Eastman Kodak Company

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May 28, 2003

Christine Todd Whitman, Administrator
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 2-Pyrrolidone

Dear Administrator Whitman:

The following comments on the 2-Pyrrolidone Consortium's (BPPB Consortium) test plan for 2-Pyrrolidone 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.

The 2-Pyrrolidone Consortium submitted its test plan on January 31, 2003 for the chemical 2-Pyrrolidone (CAS No. 616-45-5). This chemical is prepared from butyrolactone (CAS No. 96-48-0) and used most extensively as an intermediate in the production of vinylpyrrolidone but is also used as a high-boiling solvent in petroleum processing. A substantial number of physicochemical, fate, and toxicity studies have been conducted with 2-Pyrrolidone. In addition, worker exposure to this chemical in industrial applications is limited due to good industrial hygiene practices. This test plan fully utilizes existing studies, as well as other data on 2-Pyrrolidone, to fulfill all SIDS endpoints in the HPV screening program. For instance, a weight-of-evidence analysis of developmental and subchronic studies is used to meet the SIDS requirement for a reproductive toxicity study, thus avoiding a checklist approach to toxicology. This is a scientifically valid analysis and adequate for a screening level program.

We applaud the 2-Pyrrolidone Consortium's efforts and concur that no additional testing is necessary for this chemical under the HPV Challenge Program. Although the available studies on 2-Pyrrolidone do not meet all the current OECD guidelines, we commend this group for its thoughtful analysis and conclusion that additional studies will not add to our understanding of this chemical's toxicity. This approach is consistent with the EPA's stated goal of maximizing the use of existing data in order to limit additional animal testing and to avoid a mere box-checking approach to toxicology. 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

201-14529

NCIC HPV
Sent by: Mary-Beth Weaver

06/04/2003 10:21 AM

To: NCIC HPV, moran.matthew@epa.gov
cc:
cc:
Subject: Environmental Defense comments on 2-Pyrrolidone (CAS# 616-45-5)

Richard_Denison@environmentaldefense.org on 06/02/2003 02:02:55 PM

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

Subject: Environmental Defense comments on 2-Pyrrolidone (CAS# 616-45-5)

(Submitted via Internet 6/02/03 to oppt.ncic@epa.gov, hpv.chemrtk@epa.gov, boswell.karen@epa.gov, chem.rtk@epa.gov, lucierg@msn.com and erauckman@charter.net)

Environmental Defense appreciates this opportunity to submit comments on the robust summary/test plan for 2-Pyrrolidone (CAS# 616-45-5).

The test plan and robust summaries for 2-pyrrolidone (2-PO) were submitted by the 2-PO Consortium and were prepared by the Toxicology and Regulatory Affairs Group. Overall, the documents are informative and well-written. 2-PO has a very wide array of uses, including applications as a chemical intermediate, petroleum solvent, plasticizer, and ingredient in some pharmaceuticals and digital inks. Based on these applications, there are many opportunities for human and environmental exposures. It would be helpful if the sponsor provided information on the presence of 2-PO in industrial releases and additional data on the estimated or measured magnitude of human exposures from environmental or consumer sources.

The sponsor claims that existing data are adequate to fulfill requirements for all HPV endpoints. However, we do not fully agree and we recommend additional studies on the toxicity of 2-PO to aquatic invertebrates and algae. Additionally, there are some omissions in the robust summaries that raise questions regarding the adequacy of data for the reproductive toxicity endpoint. Specific comments are as follows:

1. Available data from experiments, estimations and the use of surrogates clearly indicate that 2-PO is readily biodegradable and that it should not accumulate in the environment.

2. Data presented in the robust summaries indicate that 2-PO has low acute toxicity, is not genotoxic and has low toxicity in repeat dose experiments with no apparent target organ.

3. Existing data on the toxicity to aquatic invertebrates and algae are inconsistent in that in both cases ECOSAR predictions are in dramatic conflict with experimental data. For example, ECOSAR predictions for Daphnia toxicity are 8733 mg/l whereas one experiment indicated and LD 50 of 13 mg/l. A similar wide disparity in ECOSAR predictions and experimental data occurred for algal toxicity. The sponsor has a plausible explanation for these findings based on the possibility that the 2-PO used in the experiments might have been contaminated with gamma butyrolactone, which is an intermediate in the synthesis of 2-PO. Gamma butyrolactone is highly toxic to both plants and aquatic invertebrates. However, the identities and levels of contaminants in the Z-PO experiments have not been indicated and

the algal experiments were conducted using a 2-PO sample that was 99.5% pure. For these reasons, we recommend that the sponsor conduct additional experiments on the toxicity of 2-PO to aquatic invertebrates and plants using a test substance subjected to rigorous chemical analysis.

4. The sponsor states that the existence of high-quality repeat dose and developmental toxicity studies showing no apparent effect on reproductive tract organs negates the need for a reproductive toxicity study. While we agree with this policy and the existing studies are certainly good studies, we reserve judgment at this time with respect to whether a reproductive toxicity study is needed, for the following two reasons. First, in cases where histological analysis of reproductive tract organs is used as a basis for negating the need for reproductive toxicity studies, we recommend that the list of reproductive tract tissues that were examined be listed in the robust summaries. Second, the test plan states that there are three existing developmental toxicity studies: two in rats using oral gavage were essentially negative, while the other using ip injection was apparently positive. The positive study was not made available in the robust summaries so we were not able to evaluate its quality. This study should be made available, although we do agree that the oral gavage route of exposure is a more relevant route of exposure for 2-PO.

Thank you for this opportunity to comment.

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

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

June 16, 2003

Elmer Rauckman, Ph.D., DABT
Consulting Toxicologist
BPPB Consortium
1201 Anise Court
Freeburg, IL 62243

Dear Dr. Rauckman:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for 2-Pyrrolidone posted on the ChemRTK HPV Challenge Program Web site on January 31, 2003. I commend The BPPB Consortium on behalf of the 2-Pyrrolidone Consortium for its commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its Challenge Web site, EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the enclosed comments on the HPV Challenge Web site within the next few days. As noted in the comments, we ask that The BPPB Consortium on behalf of the 2-Pyrrolidone Consortium advise the Agency, within 60 days of this posting on the Web site, of any modifications to its submission.

If you have any questions about this response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-564-7649. Submit questions about the HPV Challenge Program through the "Contact Us" link on the HPV Challenge Program Web site pages or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at tsca-hotline@epa.gov.

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

-S-

Oscar Hernandez, Director
Risk Assessment Division

Enclosure

cc: W. Penberthy
M. E. Weber

EPA Comments on Chemical RTK HPV Challenge Submission:
2-Pyrrolidone

Summary of EPA Comments

The sponsor, the 2-Pyrrolidone Consortium, submitted a test plan and robust summaries to EPA for 2-pyrrolidone (CAS No. 616-45-5) dated December 30, 2002. EPA posted the submission on the ChemRTK HPV Challenge Web site on January 31, 2003.

EPA has reviewed this submission and reached the following conclusions:

1. Physicochemical Properties and Environmental Fate. Adequate data are available for all endpoints for the purposes of the HPV Challenge Program.

2. Health Effects. Adequate data are available for all endpoints except reproductive toxicity for the purposes of the HPV Challenge Program. EPA reserves judgement on the adequacy of the reproductive toxicity data pending receipt of more details of the histopathology on reproductive organs from the submitted 90-day rat study. The submitter needs to address deficiencies in some robust summaries.

3. Ecological Effects. Adequate data are available for all endpoints 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 2-Pyrrolidone Challenge Submission

Test Plan

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

Adequate data are available for the purposes of the HPV Challenge Program.

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

Adequate data are available for the purposes of the HPV Challenge Program.

Biodegradation. EPA agrees that available data for this endpoint are adequate. While it is inappropriate to use an inherent biodegradation study to draw conclusions about ready biodegradation, and BIOWIN estimates are insufficient to adequately address this endpoint, the submitted data including the ready biodegradation study for the analogue N-methyl-2-pyrrolidone satisfy the endpoint for the purposes of the HPV Challenge Program.

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

Adequate data are available for the purposes of the HPV Challenge Program except for reproductive toxicity.

Reproductive Toxicity. EPA reserves judgement on the adequacy of available reproductive toxicity data pending receipt of more details of the histopathology on male and female reproductive organs from the submitted 90-day oral study in rats. These data, if adequate, plus data from the oral developmental toxicity study in rats will satisfy the reproductive toxicity endpoint for the purposes of the HPV Challenge

Program. The submitter needs to include all relevant data in a separate robust summary for this endpoint.

Ecological Effects (fish, invertebrates, and algae)

Adequate data are available for all ecotoxicity endpoints for the purposes of the HPV Challenge Program. However, for the acute fish toxicity study, the submitter needs to express the LC_{50} as the geometric mean of the two highest concentrations in order to be consistent with OECD Guideline 203.

The submitter also needs to address more fully and, if possible, explain the disagreement between the EC_{50} values reported for Daphnia magna (48-h EC_{50} >500 mg/L and 96-h EC_{50} >10,000 mg/L) and Daphnia pulex (48-h EC_{50} = 13.21 mg/L).

Specific Comments on the Robust Summaries

Environmental Fate

Biodegradation. The robust summary of the 2-pyrrolidone study is unclear as to whether this is an inherent or a ready biodegradation study. The methodology is stated as following the Zahn-Wellens test procedure, which is used for testing inherent biodegradation. However, the summary states that it uses "non-adapted sludge flora," which indicates a ready biodegradation study. The summary also states that "...the conditions do not meet the OECD 301 series." The OECD 301 series is for ready biodegradation and the OECD 302 series is for inherent biodegradation. The test temperature was not reported.

Health Effects

Acute Toxicity. The submitter needs to provide the following information: the length of the observation period, necropsy analyses (if performed), and a range or 95% confidence interval for the LD_{50}.

Repeated-Dose Toxicity. The submitter needs to include the magnitude of the kidney weight changes and identify the organs that were examined for gross pathology and histopathology, especially those associated with reproduction.

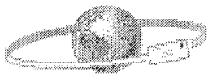
Ecological Effects

Fish and Invertebrates. The submitter needs to indicate whether the toxicity values from critical studies were based on measured or nominal concentrations and provide missing information on GLP compliance in the summary of the acute invertebrate study.

Followup Activity

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

201-14664



EJ Rauckman <erauckman@charter.net> on 08/14/2003 12:01:32 AM

To: oppt.ncic@epamail.epa.gov
cc: Jane Vergnes <JVergnes@ispcorp.com>, Christopher Bradlee <bradlec@basf-corp.com>

Subject: Revised HPV Documents for 616-45-5

Hi,

On behalf of the BPPB Consortium, I am submitting the revised Test Plan and Robust Summaries for the HPV submission of 2-Pyrrolidone (CASNO 616-45-5). The Test Plan, Robust Summaries and Cover Letter are attached as PDF documents. The cover letter addresses each of EPA's comments on the test plan and robust summaries.

Please contact me by email or phone if you have any difficultly with this transmission or have any questions.

Best regards,

//Sig//

Elmer Rauckman, PhD DABT

618-539-5280

rauckman@toxicsolutions.com



616-45-5-Cov Let. 616-45-5-Rev Test Pla 616-45-5-Rev RS.

201-14664

Elmer Rauckman, Ph.D. DABT
Toxicology and Regulatory Affairs

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Freeburg, IL 62243

Phone: (618) 539-5280 rauckman@toxicsolutions.com Fax: (618) 539-5394

13 August 2003

Ms. Marianne L. Horinko
US Environmental Protection Agency
1200 Pennsylvania Ave., N. W.
Washington, DC 20460

Re: Revision of 2-Pyrrolidone (616-45-5) Documents
Via Electronic Submission to: Oppt.ncic@epa.gov

Registered with EPA as:
BPPB Consortium, **Registration Number**

Dear Acting Administrator Horinko;

On behalf of the BPPB Consortium, Toxicology and Regulatory Affairs is hereby responding to the U.S. EPA's comments posted June 19, 2003 on the Chem-RTK HPV Challenge Web site for the Test Plan and Robust Summaries of 2-Pyrrolidone (616-45-5). The U.S. EPA's comments can be broadly grouped into two categories; testing related comments and comments pertaining to information in the Test Plan or Robust Summaries. The following are responses to the U.S. EPA's comments/questions based on these two groups:

Testing Related Issues

U.S. EPA Comment (1): EPA reserves judgment on the adequacy of available reproductive toxicity data pending receipt of more details of the histopathology on male and female reproductive organs from the submitted 90-day oral study in rats. These data, if adequate, plus data from the oral developmental toxicity study in rats will satisfy the reproductive toxicity endpoint for the purposes of the HPV Challenge Program. The submitter needs to include all relevant data in a separate robust summary for this endpoint.

BPPB Response (1): The reproductive organ histopathology in the 90-day oral study was extensive. Details of the methodology and findings were obtained and an additional robust summary was prepared addressing the histopathology of the reproductive organs. We believe that this additional information in combination with the developmental toxicity fully fills the HPV requirements for reproductive toxicity.

U.S. EPA Comment (2): For the acute fish toxicity study, the submitter needs to express the LC_{50} as the geometric mean of the two highest concentrations in order to be consistent with OECD Guideline 203.

BPPB Response (2): The robust summary was modified to express the LC50 as the geometric mean of the two highest concentrations according to the OECE 203 guidance.

U.S. EPA Comment (3):The submitter needs to address more fully and, if possible, explain the disagreement between the EC_{50} values reported for Daphnia magna (48-h EC_{50} >500 mg/L and 96-h EC_{50} >10,000 mg/L[1]) and Daphnia pulex (48-h EC_{50} = 13.21 mg/L).

BPPB Response (3): Further investigation did not identify a definitive explanation for the differences in reported EC_{50} values for these two species. On a weight of evidence basis, considering the actual data, data from similar compounds and the chemical structure, the low EC50 value for pulex seems to be an outlier. This observation was added to the Test Plan and an extensive footnote was also added providing additional rationale supporting the reliability of the Daphnia magna EC50 values.

Test Plan and Robust Summaries

U.S. EPA Comment (4):Biodegradation. The robust summary of the 2-pyrrolidone study is unclear as to whether this is an inherent or a ready biodegradation study. The methodology is stated as following the Zahn-Wellens test procedure, which is used for testing inherent biodegradation. However, the summary states that it uses "non-adapted sludge flora," which indicates a ready biodegradation study. The summary also states that "...the conditions do not meet the OECD 301 series." The OECD 301 series is for ready biodegradation and the OECD 302 series is for inherent biodegradation. The test temperature was not reported.

BPPB Response (4): As stated, the study in question was a Zahn-Wellens test for inherent biodegradation. This has been further clarified in the robust summary. The temperature was not reported in the test data available. The order of the biodegradation summaries was changed to put the critical study first.

U.S. EPA Comment (5):For the acute mammalian toxicity test, the submitter needs to provide the following information: the length of the observation period, necropsy analyses (if performed), and a range or 95% confidence interval for the LD_{50}.

BPPB Response (5): The requested information was added to the robust summary: however, as this was a limit test without mortality a 95% confidence interval cannot be calculated.

U.S. EPA Comment (6):For repeated-dose toxicity, the submitter needs to include the magnitude of the kidney weight changes and identify the organs that were examined for gross pathology and histopathology, especially those associated with reproduction.

[1] The >10,000 mg/L value in the EPA comment is apparently a typo as the reported value is 96-hr LC50 > 1,000 mg/L, not >10,000 mg/L.

BPPB Response (6): The requested information about kidney weight was added to the robust summary, as were complete lists of tissues examined at necropsy and examined microscopically. The reproductive organs were included in the lists and a more extensive description of the reproductive organ evaluation and results has been added in a separate robust summary under "fertility".

U.S. EPA Comment (7):The submitter needs to indicate whether the toxicity values from critical studies were based on measured or nominal concentrations and provide missing information on GLP compliance in the summary of the acute invertebrate study.

BPPB Response (7): The requested information was added to the robust summaries.

The Test Plan and Robust Summaries have been revised to incorporate the changes noted above. This completes the BPPB Consortium's commitment for 2-Pryollidone. Please contact me at (618) 539-5280 if you have any questions or comments.

Sincerely,

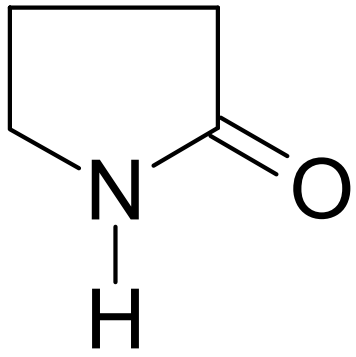
Elmer Rauckman, PhD, DABT
Consulting Toxicologist

Attachments:

Testing Plan	616-45-5-Rev Test Plan.pdf
Robust Summaries	616-45-5-Rev RS.pdf

201-14664A

2-Pyrrolidone



CAS Number 616-45-5

U.S. EPA HPV Challenge Program
Revised Submission

13 August 2003

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Submitted by:

BPPB Consortium

Prepared by:
Toxicology and Regulatory Affairs
1201 Anise Court
Freeburg IL 62243
618-539-5280

Table of Contents

Executive Overview

2-Pyrrolidone, CAS no. 616-45-5, is a cyclic amide prepared primarily from butyrolactone. It is a clear liquid with an unpleasant ammonia-like odor and a freezing point of 25° C. It has low volatility (boiling point 245 °C and vapor pressure of 0.013 hPa @ 25˚ C) and is miscible with water and most organic solvents. Its most extensive use is as a chemical intermediate but it is also used as a high-boiling solvent.

In the environment, based on physicochemical and experimental data, 2-Pyrrolidone will not bioaccumulate (Log $K_{o/w}$ = -0.71) and will distribute primarily to water where it will be subject to limited volatilization and rapid biodegradation. It is expected to react rapidly with atmospheric hydroxyl radicals with a half-life of about 11 hours. The toxicity of Propargyl alcohol to aquatic species is very low, with an LC_{50} for freshwater fish greater than 4600 mg/L and daphnia greater than 1000 mg/L.

The oral LD_{50} of 2-Pyrrolidone is very high with values of 8000 and greater than 5000 mg/kg being reported. Exposure of rats to saturated vapor for 8 hours did not produce any adverse effects and the dermal LD50 in rabbits is greater than 2000 mg/kg.

A modern subchronic drinking water study of 2-Pyrrolidone showed low repeated-dose toxicity with a 90-day NOAEL of 2400 ppm and a LOAEL of 7200 ppm in drinking water. The kidneys many have been affected but no target organs were identified by histopathological examination.

Adequate *in vitro* tests of genetic toxicity for 2-Pyrrolidone are available. A *Salmonella typhimurium* reverse mutation assay shows lack of mutagenic activity in the presence or absence of metabolic activation and a guideline cytogenetics study using human lymphocytes displayed a lack of genotoxic activity in the presence or absence of metabolic activation.

Developmental toxicity has been investigated using an OECD 414 Guideline study. The results of this investigation conducted in rats by oral gavage at 0, 190, 600 or 1900 mg/kg-day indicate that 2-P affects the conceptus only at doses that exceed the maternally toxic level. The developmental NOAEL was found to be 600 mg/kg-day while the maternal NOAEL was 190 mg/kg-day.

The combination of the negative developmental toxicity study with a robust subchronic study in which specific damage to reproductive organs was carefully evaluated and not observed fulfills the current requirement for reproductive toxicity information.

It is concluded that the available information adequately fills all the data elements of the HPV. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, conduct of additional similar studies would not add significantly to our understanding of this material’s hazard.

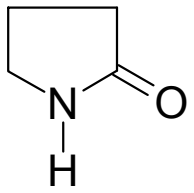
Testing Plan and Rationale

Testing Plan in Tabular Format

CAS Number 616-45-5 2-Pyrrolidone	Information Available?	OECD Study ?	GLP Study?	Supporting Information?	Estimation Method?	Acceptable?	Testing Recommended?	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	N	N	Y	N	
Boiling Point	Y	N	N	N	N	Y	N	
Vapor Pressure	Y	N	N	Y	N	Y	N	
Partition Coefficient	Y	Y	N	Y	N	Y	N	
Water Solubility	Y	N	N	Y	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	N	Y	Y	N	
Water Stability	Y	N	N	Y	Y	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	Y	N	N	Y	N	Y	N	
Ecotoxicity								
96-Hour Fish	Y	Y	N	Y	N	Y	N	
48-Hour Invertebrate	Y	Y	N	Y	N	Y	N	
72-Hour Algae	Y	Y	N	Y	N	Y	N	
Toxicity								
Acute	Y	N	N	Y	N	Y	N	
Repeated Dose	Y	Y	Y	N	N	Y	N	
Genetic Toxicology *in vitro*	Y	N	Y	Y	N	Y	N	
Genetic Toxicology *in vivo*	Y	N	Y	Y	N	Y	N	
Reproductive	Y	N	N	Y	N	Y	N	
Developmental	Y	Y	Y	Y	N	Y	N	

Introduction

2-Pyrrolidone, CAS no. 616-45-5, is a cyclic amide prepared primarily from butyrolactone by a Reppe process (1). It is a clear liquid (above 25° C) with an unpleasant ammonia-like odor. It has low volatility and is miscible with water and most organic solvents. Its most extensive uses are as an intermediate in the manufacture of N-methylpyrrolidone, vinylpyrrolidone, polyvinylpyrrolidone and polypyrrolidone with over 95% of the 2-Pyrrolidone production going into vinylpyrrolidone (2). It is used as a high-boiling solvent in petroleum processing and acrylonitrile manufacture. It also finds application as a solvent for polymers, sorbitol, glycerol, iodine and sugars. Some is used as a plasticizer and coalescing agent for polymer emulsion coatings such as floor polishes. Another application is as humectant and co-solvent for digital printing inks. Its exceptional solvent properties make it very useful for the solubilization of complex organic material in water. Although it is an excellent solvent, the somewhat labile proton on the nitrogen limits its applications as an aprotic solvent. Its structure is shown below:



2-Pyrrolidone is also known as:

- 4-Aminobutyric acid lactam
- Gamma-aminobutyric lactam
- Gamma-aminobutyrolactam
- Butanoic acid, 4-amino-, lactam
- Butyrolactam
- Gamma-butyrolactam
- 2-Ketopyrrolidine
- 2-Oxopyrrolidine
- 2-Pyrol
- Apha-pyrrolidinone

The chemical and physical properties of 2-Pyrrolidone make it a unique solvent for certain applications and a useful chemical intermediate. There are several reports in the open literature of its utility as a skin-penetration enhancer with potential applications in transdermal drug delivery. This property and potential application seems

to be a function of the physicochemical properties of this solvent and not a specific chemical reactive property. Another use in the pharmaceutical industry is in the production of pyrrolidone nootropics including piracetam (2).

Exposure in industrial applications is limited by process controls, protective equipment, a very low vapor pressure and excellent warning properties due to its objectionable odor. No occupational exposure level set by a governmental agency could be located for 2-Pyrrolidone. Use as a humectant and co-solvent in digital inks may result in a low-level of inhalation exposure by consumers limited by the very low quantities of inks used by digital printing devices.

Several physicochemical, fate and toxicity studies have been conducted on 2-Pyrrolidone. These studies are briefly reviewed in this testing rationale document, which also describes how these studies meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (USEPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries or given as shorter summaries using the IUCLID format. The available data set satisfactorily fulfills the data requirements for the EPA HPV Program. The majority of data elements are filled by high-reliability studies on 2-Pyrrolidone. Where direct data are not available or data are sparse, surrogates and estimations are used to fill the data element. This activity is encouraged by the U.S. EPA and other regulatory authorities to avoid unnecessary testing and animal usage.

Physicochemical Data

Physicochemical data for 2-Pyrrolidone are available from the literature and manufacturer's information.

Table 1: Physicochemical Properties of 2-Pyrrolidone

Melting Point	25˚ C (3)
Boiling Point	245° C @ 1010 hPa (4)
Vapor Pressure	0.013 hPa @ 25˚ C (5)
Partition Coefficient	Log $K_{o/w}$ = -0.71 (6)
Water Solubility	Soluble in all proportions (7)

These properties indicate that above 25° C, 2-Pyrrolidone is slightly volatile liquid with high water solubility. The value of the partition coefficient suggests that 2-Pyrrolidone will partition preferentially into water and, therefore, has little potential for bioaccumulation.

Recommendation: No additional physicochemical studies are recommended. The available data fill the HPV required data elements.

Environmental Fate and Pathways

Biodegradation potential has been determined using a Zahn Wellens test. In this DOC removal test, DOC was 80% eliminated after 5 days of incubation (8). Although this only definitively shows "inherent biodegradability" the speed of removal and completeness (99% at 9 days) suggest that this material is easily biodegraded by non-adapted bacteria. Using BIOWIN 4.00, it can be estimated that 2-Pyrrolidone is readily biodegradable with quantitative estimates suggesting a high likelihood that it should be considered "readily biodegradable (9). Furthermore, the analog and surrogate compound, N-Methyl-2-pyrrolidone (NMP) has been demonstrated to be readily biodegradable in the MITI test (10). Comparative estimation using BIOWIN 4.00 suggests that NMP is likely to be slightly more resistant to aerobic biodegradation than 2-Pyrrolidone, although NMP still is indicated by BIOWIN to be readily biodegradable. The information that NMP biodegradation is correctly predicted as readily biodegradable by BIOWIN, and the strong structural similarity between the two compounds, validates the BIOWIN estimate for 2-Pyrrolidone.

Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The program produced an estimated rate constant of 11.9 E-12 cm^3/molecule-sec. Using the default atmospheric hydroxyl radical concentration in APOWIN and the estimated rate constant for reaction of 2-Pyrrolidone with hydroxyl radical, the estimated half-life of 2-Pyrrolidone vapor in air is approximately 10.75 hours (see accompanying robust summary).

Water stability has not been quantitatively determined for 2-Pyrrolidone. Quantitative stability determinations (e.g. OECD 111) are considered unnecessary for compounds containing only non-hydrolysable groups, as the SIDS manual states that consideration should be given to using an estimation method. There is no evidence available that 2-Pyrrolidone is unstable in water, although it has a potentially hydrolysable amide group, amides are considered resistant to hydrolysis at environmental pH values and require strong base or acid to accomplish hydrolysis. Vollhardt states: "Amides are the least reactive of the carboxylic derivatives, mainly because of the extra resonance capacity of the nitrogen lone electron pair. As a consequence, their nucleophilic addition-eliminations require relatively harsh conditions. For example, hydrolysis occurs only on prolonged heating in strongly acidic or basic water"(11). The HYDOWIN program recognized this when an estimate of hydrolysis was attempted. The HYDROWIN output was that the compound had an amide group and the hydrolysis rate was extremely slow, the HYDROWIN program estimated the half-life in water greater than one year (12). This estimated is confirmed by the review of Harris, who notes that the mean hydrolytic half-life for a series of amides is in the range of 300 years (13). In addition, this is a cyclic amide in a 5-membered ring, which is generally the ring size showing the least strain and, hence making ring opening a less favored occurrence increasing resistance to hydrolysis.

Theoretical Distribution (Fugacity) of 2-Pyrrolidone in the environment was estimated using the MacKay EQC level III model with standard defaults in EPIWIN v 3.05 but using the measured vapor pressure of 0.013 hPa and the measured log $K_{o/w}$ (14). The results for distribution using a model calculated $K_{o/c}$ (adsorption coefficient based on organic carbon content) of 0.0799 and equal initial distribution to air, water and soil are:

- Air 0.4 %
- Water 46.5 %
- Soil 53.0 %
- Sediment 0.08 %

Recommendation: No additional fate studies are recommended. The available data fill the HPV required elements.

Ecotoxicity

A recent GLP guideline (OECD 203) study of acute fish toxicity using measured concentrations of 2-Pyrrolidone is available demonstrating low hazard to zebra fish after 96 hours of exposure. The test material stability in the dilution water with fish was very good over the 96-hour period. Daphnia studies indicate an EC_{50} greater than 1000 mg/L in one test, greater than 500 mg/L in another guideline-like study and a report of an EC_{50} values less than 20 mg/L. The two higher EC50 values were obtained in studies with *D. magna*, while the 13.2-mg/L value was obtained for *D. pulex*. The low value for *D. pulex* is not consistent with the weight of evidence considering the data for *D. magna* and *D. pulex*, aquatic toxicity data for similar compounds and predictions based upon the chemical structure.[a] Although these experimental data give differing results, the weight of evidence indicates a low aquatic hazard. Other invertebrates, specifically, flatworms and snails, showed no effects in limit tests at 112 mg/L. Algae growth inhibition, according to a guideline study, has an EC_{50} of about 84 mg/L after 96-hours. These values with references are shown in the table. ECOSAR estimates, using the neutral organic model, are also given in the table below for comparison. In addition, a bacterial growth inhibition test using *Pseudomonas putida* resulted in an EC_{50} of 9368 mg/L, with lower concentrations showing stimulation of bacterial growth (15).

Table 2: Comparative Aquatic Toxicity of 2-Pyrrolidone

	Reported Values	ECOSAR Prediction
Fish, 96 hour LC_{50}	> 4600 mg/L (16)	9566 mg/L*
Daphnia, 48 hour EC_{50}	> 500 mg/L (17) > 1000 mg/L (18) = 13.2 mg/L (19)	8733 mg/L*
Algae, 96 hour EC_{50}	= 84 mg/L (20)	4777 mg/L*

* Estimated using ECOSAR (21)

Un-validated, but multiple, study results reported in IUCLID 2000 (22) indicate that the analog 1–methyl–2–pyrrolidone has low acute toxicity to fish, invertebrates and algae (short-term LC_{50} or EC_{50} values >500 mg/L). This lends support to the higher values for the LC_{50} and EC_{50} values of 2-Pyrrolidone that have been reported. The reason some investigations have found higher degrees of toxicity is unknown but a reasonable speculation might be that the samples tested were contaminated with more toxic agents. For example, it is known that γ-Butyrolactone which is one of the primary starting materials for 2-Pyrrolidone is more toxic to fish and daphnids.

[a] The 13.8 mg/L value for *Daphnia pulex* is thought to be an outlier for invertebrate toxicity of 2-Pyrrolidone as this same report provided an EC_{50} for N-Methylpyrrolidone of 2.1 mg/L for *Daphnia pulex* when studies have shown that the EC_{50} for N-Methylpyrrolidone to *Daphnia magna* is greater than 1000 mg/L and other crustacea show similar sensitivity to N-Methylpyrrolidone (IUCLID-2000 record for 872-50-4). A literature search was conducted for species sensitivity of these two daphnids to chemicals. Publications found in TOXLINE indicated that both species have similar sensitivity to most chemicals. The weight of evidence favors the higher EC_{50} values reported for the *D magna*.

Likewise, aliphatic amines, which are potential side products from 2-Pyrrolidone manufacture, typically have LC and EC_{50} values in a range where contamination of a sample might result in a low EC_{50}.

Recommendation: No additional ecotoxicity studies are recommended. The available data fill the HPV required endpoints. Although experimental data give differing results, the weight of evidence indicates low aquatic hazard. This information coupled with the information that 2-Pyrrolidone is biodegraded easily in the environment and has a low log $K_{o/w}$ constant reduce the concern level for potential environmental hazard. Conduct of additional studies would not add significantly to our understanding of this material's toxicity and it is recommended that no additional ecotoxicity studies be conducted.

Health Effects

Acute Toxicity

Oral Exposure

Multiple determinations of the oral LD_{50} of 2-Pyrrolidone have been reported (23) and the studies universally indicate a low order of acute oral toxicity for this material. Two robust summaries have been prepared from BASF study reports. One indicted an LD_{50} of approximately 8000 mg/kg-bw (24) and the other was a limit test at 5000 mg/kg-bw in which there were no mortalities or adverse clinical signs except for transient loss in male body weights (25).

Inhalation Exposure

It has been reported that there were no deaths when rats were exposed to saturated vapor of 2-Pyrrolidone for 8 hours (26). The actual concentration was not measured but based on the vapor pressure at 30°C the vapor concentration is calculated to be in the range of 15-20 ppm.

Dermal Exposure

A guideline (OECD 402) limit study has indicated that the dermal LD_{50} of 2-Pyrrolidone in rabbits is greater than 2000 mg/kg-bw (27).

Recommendation: No additional acute toxicity studies are recommended. The available data fill the HPV required endpoints for acute toxicity. Although the available studies do not meet the requirements of the current OECD guidelines in all cases, the weight of evidence shows that the oral and dermal toxicity is very low. Likewise, the limited study of acute saturated vapor inhalation provides important and scientifically defensible information about vapor toxicity. Conduct of additional studies would not add significantly to our understanding of this material's toxicity and it is recommended that no additional acute toxicity studies be conducted.

Repeat Dose Toxicity

Oral Exposure

A guideline-glp 90-day study in rats has been conducted. In this study, 2-Pyrrolidone was administered to groups of 10 male and 10 female Wistar rats at doses of 0; 600; 2,400; 7,200 and 15,000 ppm in the drinking water over a period of 3 months (28). No animals died nor were any adverse clinical signs of exposure reported. In the high-dose group, food and water consumption, and body-weight gain were reduced for males and females; kidney weights for males and females were increased; other minor treatment related effects were in prolonged prothrombin times and decreased serum protein, globulins, creatinine and triglycerides. At 7,200 ppm, water

consumption was reduced in rats of each sex; food consumption and body weight gain were reduced only for females; kidney weights for males were increased; other minor treatment related effects were decreased serum total protein for females and decreased creatinine in both sexes. The 2,400 ppm dose was a NOAEL. Gross pathology, organ weight determination and full histopathology were conducted on all animals. No treatment-related histopathologic effects were observed.

Recommendation: No additional repeated-dose studies are recommended. The available data conducted by OECD Guidelines and under GLP fill the HPV required endpoint for repeated-dose toxicity.

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points: generally one sensitive to point mutation and one sensitive to chromosomal aberrations. In the case of this material, adequate tests have been conducted that cover both of these endpoints.

Genetic Toxicology in vitro

Adequate *in vitro* tests of genetic toxicity for 2-Pyrrolidone are available. A *Salmonella typhimurium* reverse mutation assay shows lack of mutagenic activity in the presence or absence of metabolic activation (29). Likewise, a guideline cytogenetics study using human lymphocytes displayed a lack of genotoxicity activity in the presence or absence of metabolic activation (30).

Genetic Toxicology in vivo

Mammalian genotoxicity was assessed *in vivo* using the Mouse Micronucleus Test. In this OECD-Guideline-474 study, a single i.p. dose of 2-Pyrrolidone did not result in an increase in normochromatic erythrocytes containing micronuclei. It was concluded that the test material did not show genotoxic activity in this system (31).

Recommendation: The SIDS requirement for genetic testing has been met as assays sensitive to both point mutation and to clastogenic effects have been conducted using acceptable protocols. No additional genotoxicity testing is recommended.

Reproductive Toxicity

The combination of the negative developmental toxicity study (32) with a robust subchronic study (28) showing that, even at systemically toxic doses, there is no specific damage to reproductive organs of males or females, fulfills the current requirement for reproductive toxicity information. As part of the subchronic study, a detailed gross and microscopic examination of male and female reproductive organs was conducted. The extent of this investigation was sufficient to prepare a robust summary (section on fertility) providing the procedures and results of this detailed investigation. No effects on reproductive organs were detected that indicate the test material will affect fertility.

Recommendation: No additional reproductive testing is recommended, as the available data are sufficient to assess the reproductive toxicity of this material.

Developmental Toxicity

A modern OECD 414 Guideline study has been conducted with 2-Pyrrolidone. The results of this investigation conducted in rats by oral gavage at 0, 190, 600 or 1900 mg/kg-day indicate that 2-Pyrrolidone is embryotoxic at doses that exceed the maternally toxic level. The developmental NOAEL was found to be 600 mg/kg-day while the maternal NOAEL was 190 mg/kg-day. Even at the maximum dose level of 1900 mg/kg-day the developmental toxicity was not severe (32). This result is supported by an older single-dose-level teratology study at about 1900 mg/kg-day in the same strain of rat by oral gavage. In this study, 25 presumed-pregnant dams were treated from day 6 to 15 of gestation. Fetuses were delivered by Caesarean section on GD-20 and examined for external, visceral and skeletal abnormalities. No differences were reported between the control and treated animals (33). A mouse teratology study using i.p. injection has also been conducted. Some degree of developmental toxicity was reported in this study but the effect was considered due to stress on the animals from the i.p. injections (34). The proposed explanation is consistent with mouse physiology; moreover, the route of exposure is inappropriate in a consideration of hazard or risk assessment.

Taken together, the weight of evidence from these developmental toxicity studies indicates a low developmental toxicity hazard for 2-Pyrrolidone.

Recommendation: No additional developmental toxicity testing is required as the available data are sufficient to assess the developmental toxicity of this material.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, it is concluded that the available information fills all of the requirements for physicochemical parameters, fate information, aquatic toxicity and mammalian toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, taken together the information provides a reliable hazard assessment. Conduct of additional studies would not add significantly to our understanding of this material's toxicity.

References

1 O'Neil, MJ (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Thirteenth edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001

2 Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VHC Verlag GmbH, 2002

3 Flick, E.W. (ed.). Industrial Solvents Handbook 4 th ed. Noyes Data Corporation., Park Ridge, NJ., 1991. 918, as cited in Hazardous Substance Data Base, NLM, Revison of 8-6-2002

4 Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996. 1378

5 Daubert, T.E. and Danner, R.P. Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. Design Institute For Physical Property Data, American Institute Of Chemical Engineers. Hemisphere Pub. Corp., New York, NY., 5 Vol, 1997

6 BASF AG, Analytisches Labor; Unpublished Study (J.Nr.129300/04 vom 14.06.88)

7 Riddick, J.A.; Bunger, W.B.; and Sakano, T.K. Organic Solvents: Physical Properties And Methods Of Purification. Techniques Of Chemistry. 4th Ed. New York, NY: Wiley-Interscience. 2: Pp.1325, 1986 (as cited in CIS 4-2002)

8 BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung (Pyrrolidon dest., 1977) see robust summary.

9 BIOWIN 4.00 SRC, See Robust Summary for details of method and results of modeling.

10 Chem Inspect Test Inst; Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan; Published by Japan Chemical Industry Ecology-Toxicology & Information Center. ISBN 4-89074-101-1 p. 5-5 (1992)

11 Vollhardt, K. "Organic Chemistry" WH Freeman and Co, New York, 1987, p 815.

12 HYDROWIN v1.67, Syracuse Research Corporation, Syracuse NY, available through the U.S. EPA.

13 J.C. Harris in Lyman W, Reehl, W and Rosenblat, D. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washingotn D.C. 1990, page 7-6

14 EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

15 BASF AG: Labor Okologie, unpublished study, 28.06.88

16 BASF AG: Abt. Toxikologie, unpublished report, (92/14), 01.08.1995

17 BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0701/88)

18 Submission to U.S. EPA: Raw data for ecotoxicity information on 2-Pyrrolidinone (CAS Reg No 616-45-5), with cover letter dated 01/29/86 Source: EPA/OTS; Doc #FYI-OTS-0794-1152 Submitted by Eastman Kodak Company

19 Perry, C.M., Smith,S.B. Toxicity of Six Heterocyclic Nitrogen Compounds to Daphnia pulex. Bull. Environ. Contam. Toxicol.41, 604-608, (1988)

20 BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0701/88, Fa.Noack)

21 ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

22 ECB IUCLID 2000, 1–Methyl–2–pyrrolidone, 19-Feb-2000, ECB

23 ECB IUCLID (2000) document for 616-45-5 2-Pyrrolidone 18-FEB-2000, ECB

24 BASF AG, Abteilung. Toxikologie, unveroeffentlichte Untersuchung,(XI/407), 07.11.1961

25 BASF AG, Abteilung Toxikologie; unveroeffentliche Untersuchungen (79/409), 09.04.1981

26 BASF AG: Abt. Toxikologie, unveroeffentlichte Untersuchung,(XI/407), 07.11.1961

27 MB Research Laboratories Inc project number MB-92-1432 Sponsored by International Specialty Products, 4/29/1992.

28 BASF AG, Report of the Subchronic oral toxicity with 2-Pyrrolidone in Wistar rats, 3-month drinking water, Project No. 52S0014/92038 June 4, 1998

29 Jagannath, D.R., Mutagenicity Test on 2-Pyrrolidone in the Ames Salmonella/Microsome Reverse Mutation Assay, Final Report, Hazleton Labs, GAF Sponsor April 24, 1987.

30 BASF AG, Abt. Toxikologie, unpublished study report (86/286), 26.11.1987

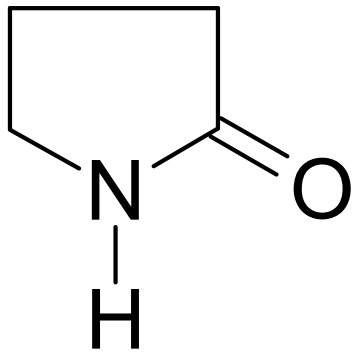
31 BASF AG, Abteilung Toxikologie; unpublished report. Cytogenetic Study In Vivo of Pyrrolidon-2 in Mice, Micronucleus test. (92/1491), 28.06.93

32 Bio-Research Laboratories Inc, An Oral Teratoloty Study of 2-Pyrrolidone in the Rat. Project # 83880, Dec. 19, 1990 Sponsored by GAF Chemicals and BASF AG

33 BASF AG, Abt. Toxikologie, unveroeffentlichte Untersuchung (XIX/421), 04.08.1971

34 BASF AG, Abteilung Toxikologie; unveroeffentliche Untersuchung (XIX/421), 29.05.1970

2-Pyrrolidone



CAS Number 616-45-5

Existing Chemical	:	ID: 616-45-5
CAS No.	:	616-45-5
EINECS Name	:	2-pyrrolidone
EC No.	:	210-483-1
TSCA Name	:	2-Pyrrolidinone
Molecular Formula	:	C4H7NO

Producer related part

Company	:	Toxicology and Regulatory Affairs
Creation date	:	06.10.2002

Substance related part

Company	:	Toxicology and Regulatory Affairs
Creation date	:	06.10.2002
Status	:	
Memo	:	
Printing date	:	13.08.2003
Revision date	:	
Date of last update	:	13.08.2003

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)	:	

1.0.1 APPLICANT AND COMPANY INFORMATION

Type : lead organisation
Name : Toxicology and Regulatory Affairs
Contact person : Elmer Rauckman PhD DABT
Date :
Street : 1201 Anise Court
Town : 62243 Freeburg, IL
Country : United States
Phone : 618-539-5280
Telefax : 618-539-5394
Telex :
Cedex :
Email : rauckman@toxicsolutions.com
Homepage : toxicsolutions.com

Remark : Participating Members of Consortium

BASF Corporation
International Specialty Products

31.12.2002

1.2 SYNONYMS AND TRADENAMES

2-Ketopyrrolidine
08.12.2002

2-Oxopyrrolidine
08.12.2002

2-Pyrol
08.12.2002

4-Aminobutyric acid lactam
08.12.2002

Apha-pyrrolidinone
08.12.2002

Butanoic acid, 4-amino-, lactam
08.12.2002

Butyrolactam
08.12.2002

Gamma-aminobutyric lactam
08.12.2002

Gamma-butyrolactam
08.12.2002

2.1 MELTING POINT

Value : = 25 °C

Test substance :
2-Pyrrolidone CAS No. 616-45-5
Reliability : (2) valid with restrictions
2 Handbook Value
Flag : Critical study for SIDS endpoint
06.10.2002 (21)

2.2 BOILING POINT

Value : = 245 °C at 1010 hPa
Decomposition :
Method :
Year :
GLP : no data
Test substance :

Test substance : CAS No. 616-45-5 2-Pyrrolidone
Reliability : (2) valid with restrictions
Handbook values are assigned 2
Flag : Critical study for SIDS endpoint
06.10.2002 (16)

2.3 DENSITY

Type : density
Value : = 1.116 g/cm³ at 25 °C
Method :
Year :
GLP : no data
Test substance :

Test substance : CAS No. 616-45-5 2-Pyrrolidone
Reliability : (2) valid with restrictions
2 Handbook Value
Flag : Critical study for SIDS endpoint
06.10.2002 (16)

2.4 VAPOUR PRESSURE

Value	:	= .013 hPa at 25 °C
Decomposition	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	
Remark	:	Given in reference as 0.00949 mm. Converted to hPa by multiplying by 1.33 hPa/mm Supported by IUCLID 2000 value of 0.04 hPa at 20 C as referenced in BASF AG, Sicherheitsdatenblatt Pyrrolidon dest. (28.06.1993)
Reliability	:	(2) valid with restrictions 2 Handbook Value
Flag	:	Critical study for SIDS endpoint

31.12.2002 (18)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	= -.71 at 25 °C
pH value	:	
Method	:	OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year	:	
GLP	:	no data
Test substance	:	
Method	:	Approximately 25 ml each of water and 1-octanol were mixed in a shake flask with 0.063, 0.137 or 0.166 grams of test substance in three separate trials at 25 deg C. After separation of the layers, the test substance was determined in quadruplicate in each phase with using gas chromatography. The mean P(OW) values for each of the three trials were 0.193, 0.193 and 0.206. These values were averaged and the log was determined to give a mean Low K0/w of -0.71
Remark	:	SRC Physical Properties Data Base lists result 0r -0.85 as published by Sasaki,H et al. (1991). EPIWIN, Log Kow (KOWWIN v1.66 estimate) = -0.32 based on smiles structure.
Test substance	:	2-Pyrrolidone CAS No. 616-45-5
Reliability	:	(1) valid without restriction 1, Modern guideline study
Flag	:	Critical study for SIDS endpoint

31.12.2002 (6)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= at °C
pH value	:	= 10 - 11
concentration	:	100 g/l at 20 °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	
Remark	:	pH of solution is from: BASF AG, Sicherheitsdatenblatt Pyrrolidon dest. (28.06.1993)
Result	:	Miscible
Test substance	:	CAS No. 616-45-5 2-Pyrrolidone
Reliability	:	(2) valid with restrictions 2 Handbook value
Flag	:	Critical study for SIDS endpoint

06.10.2002 (26)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	.000000000012 cm³/(molecule*sec)
Degradation	:	ca. 50 % after 10.8 hour(s)
Deg. product	:	
Method	:	
Year	:	2002
GLP	:	
Test substance	:	

Result :

SMILES : C1CCC(=O)N1
CHEM : 2-Pyrrolidone
MOL FOR: C4 H7 N1 O1
MOL WT : 85.11
- SUMMARY (AOP v1.90): HYDROXYL RADICALS --------
Hydrogen Abstraction = 6.4334 E-12 cm3/molecule-sec
Reaction with N, S and -OH = 5.5000 E-12 cm3/molecule-sec
Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds = 0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings = 0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec

OVERALL OH Rate Constant = 11.9334 E-12 cm3/molecule-sec
HALF-LIFE = 0.896 Days (12-hr day; 1.5E6 OH/cm3)
HALF-LIFE = 10.756 Hrs

Source	:	Toxicology and Regulatory Affairs
Test substance	:	CAS No. 616-45-5 2-Pyrrolidone
Reliability	:	(2) valid with restrictions Calculated by acceptable method
Flag	:	Critical study for SIDS endpoint

08.12.2002 (19)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	> 1 year at 25 °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	
Year	:	2002
GLP	:	no
Test substance	:	

Method :

Estimation using HYDROWIN 1.67.
Input was SMILES notation: C1CCC(=O)N1

Remark	:	Furthuer supports comes from the "Handbook of Chemical Property Estimation Methods" (2) in which is it is indicated that the mean hydrolytic half-life for a series of amides is in the range of 300 years (2) J.C. Harris in Lyman W, Reehl, W and Rosenblat, D. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washingotn D.C. 1990, page 7-6 This estimated is supported by the known properties of amides. For example in the textbook "Organic Chemistry" (1), Vollhardt states that "Amides are the least reactive of the carboxylic derivatives, mainly because of the extra resonance capacity of the nitrogen lone electron pair. As a consequence, their nucleophilic addition-eliminations require relatively harsh conditions. For example, hydrolysis occurs only on prolonged heating in strongly acidic or basic water" (1) Vollhardt, K. "Organic Chemistry" WH Freeman and Co, New York, 1987, p 815.
Result	:	HYDROWIN Program (v1.67) Results: ========== SMILES : C1CCC(=O)N1 CHEM : 2-Pyrrolidone MOL FOR: C4 H7 N1 O1 MOL WT : 85.11 ---- HYDROWIN v1.67 Results ---------- AMIDE: -N-C(=O)-C- Compound has an amide group; C=O located at SMILES atom #4 Hydrolysis Rate Extremely Slow or t1/2 > 1 Year
Source	:	Toxicology and Regulatory Affairs
Test substance	:	2-Pyrrolidone CAS No. 616-45-5
Reliability	:	(2) valid with restrictions Estimated using an acceptable method with comfirmation from both chemical principles and experimental data on surrogate compounds.
Flag	:	Critical study for SIDS endpoint
30.11.2002		(20)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other: all
Air	:	% (Fugacity Model Level I)
Water	:	% (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Method	:	other
Year	:	2002
Method	:	Determined using the Level 3 EQC Model found in EPIWIN 3.05. Actual values were used for measured physicochemical parameters. The

degredation times applied using the BIOWIN were validated by experimental data on the test substance and/or surrogate compounds

Result :

Level III Fugacity Model (Full-Output):
==========

Chem Name : 2-Pyrrolidone
Molecular Wt: 85.11
Henry's LC : 1.44e-008 atm-m3/mole (Henrywin program)
Vapor Press : 0.00949 mm Hg (user-entered)
Log Kow : -0.71 (user-entered)
Soil Koc : 0.0799 (calc by model)

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.403	21.5	1000
Water	46.5	360	1000
Soil	53	360	1000
Sedimet	0.0776	1440	0

	Fugacity (atm)	Reaction (kg/hr)	Advect (kg/hr)	Reaction (percent)	Advection (percent)
Air	1.36e-011	153	47.4	5.09	1.58
Water	4.62e-013	1050	547	35.1	18.2
Soil	1.94e-011	1200	0	40	0
Sed	3.85e-013	0.439	0.018	0.0146	0.00061

Persistence Time: 392 hr
Reaction Time: 489 hr
Advection Time: 1.98e+003 hr
Percent Reacted: 80.2
Percent Advected: 19.8

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
Air: 21.51
Water: 360
Soil: 360
Sediment: 1440
Biowin estimate: 2.957 (weeks)

Advection Times (hr):
Air: 100
Water: 1000
Sediment: 50000

Source :

Calculated by Toxicology and Regulatory Affairs, 2002

Test substance :

CAS No. 616-45-5 2-Pyrrolidone

Reliability : (1) valid without restriction
Calculated by an acceptable method using measured physicochemical parameters.

31.12.2002 (19)

3.3.2 DISTRIBUTION

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge, domestic
Contact time	:	28 day(s)
Degradation	:	= 73 (±) % after 28 day(s)
Result	:	readily biodegradable
Deg. product	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS
Method	:	Japanese MITI test
Remark	:	Surrogate material
Test substance	:	1-Methyl-2-pyrrolidinone CASNO 872-50-4 Surrogate material
Reliability	:	(2) valid with restrictions Published study result
Flag	:	Critical study for SIDS endpoint

03.08.2003 (17)

Type	:	aerobic
Inoculum	:	
Contact time	:	
Degradation	:	(±) % after
Result	:	readily biodegradable
Deg. product	:	
Method	:	other: estimation
Year	:	
GLP	:	
Test substance	:	
Method	:	The structure was run through BIOWIN 4.00, as found in EPIWIN 3.05. This software predicts, with excellent accuracy, the ease and relative rate of aerobic biodegredation. Estimates are primarily based on a fragment approach.
Remark	:	This estimate is supported by the high rate of biodegredation observed in the Zahn Wellens procedure (BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung (Pyrrolidon dest., 1977)) and the ready biodegredability of the N-methyl derivitive (NMP, see HSDB) which, based on judgement and BIOWIN modeling, is expected to be slightly more difficult to biodegrade than 2-Pyrrolidone.
Result	:	SMILES : C1CCC(=O)N1 CHEM : 2-Pyrrolidone MOL FOR: C4 H7 N1 O1 MOL WT : 85.11 BIOWIN v4.00 Results Linear Model Prediction : Biodegrades Fast Non-Linear Model Prediction: Biodegrades Fast

Ultimate Biodegradation Timeframe: Weeks
Primary Biodegradation Timeframe: Days
MITI Linear Model Prediction : Biodegrades Fast
MITI Non-Linear Model Prediction: Biodegrades Fast

LINEAR BIODEGRADATION PROBABILITY 0.9172
NON-LINEAR BIODEGRADATION PROBABILITY 0.9889

MITI LINEAR BIODEGRADATION PROBABILITY 0.6448
MITI NON-LINEAR BIODEGRADATION PROBABILITY 0.8408

A Probability Greater Than or Equal to 0.5 indicates --> Readily Degradable
A Probability Less Than 0.5 indicates --> NOT Readily Degradable

SURVEY MODEL - ULTIMATE BIODEGRADATION 2.9569
SURVEY MODEL - PRIMARY BIODEGRADATION 3.9304

Interpretation, Primary & Ultimate:
Result Classification:
5.00 -> hours
4.00 -> days
3.00 -> weeks
2.00 -> months
1.00 -> longer

Test substance :
2-Pyrrolidone CAS No. 616-45-5

Reliability : (2) valid with restrictions
Estimated using an acceptable method.

31.12.2002

Type : aerobic
Inoculum : other: activated sludge, non-adapted
Contact time :
Degradation : > 90 (±) % after 9 day(s)
Result :
Kinetic of testsubst. : 1 day(s) = 5 %
5 day(s) = 80 %
7 day(s) = 89 %
9 day(s) = 99 %
%

Method : This Inherent Biodegradation test followed the Zahn-Wellens procedure.

Triplicate determinations were made using the test substance at a final concentration of about 500 mg/L and in 2 L of culture containg 100 ml of non-adapted sludge.

Elimination was determined by measuring total organic carbon (TOC) at 0 and 3 hours; and at 1, 5, 7, and 9 days after start of the test.

The methodology follows the Zahn Wellens test procedure.

Remark :

Although the conditions do not meet the OECD 301 series, the results clearly demonstrate that non-adapted sludge flora are capable of fully degrading the test material in a short time.

Technically, this test only indicates inherent biodegradation; however, the rapidity of the biodegradation is consistent with a "readily biodegradable" material.

Test substance :

2-Pyrrolidone, Distilled

Conclusion :

The test material is considered "inherently biodegradable" showing rapid biodegredation.

Reliability : (2) valid with restrictions
The raw data for this triplicate determination was available for review; although some details were missing the method is scientifically defensible.

03.08.2003 (7)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 4600 measured/nominal
LC0	:	= 4600 measured/nominal
LC50	:	= 6800 measured/nominal
LC100	:	= 10000 measured/nominal
Limit test	:	
Analytical monitoring	:	yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	
GLP	:	yes
Test substance	:	

Method :

METHOD: Followed standard laboratory protocol for OECD 203 (April 1984).

DETAILS OF TEST: Static

DILUTION WATER SOURCE: Municipal water, carbon treated

DILUTION WATER CHEMISTRY: pH 8.0-8.6, total hardness about 2.5 mmol/L, acid capacity about 5.5 mmol/L, TOC not given, TSS not given.

STOCK AND TEST SOLUTION PREPARATION: Test substance added neat to test water 20 minutes before placing fish in aquaria.

VEHICLE/SOLVENT AND CONCENTRATIONS: Dilution water, concentrations 0, 50, 100, 1000, 2150, 4640, 10000 mg/L

STABILITY OF THE TEST CHEMICAL SOLUTIONS: Assured by analytical determination

EXPOSURE VESSEL: All-glass aquaria, 30 x22 x 24 cm, containing 10 L water and filled to a depth of about 17 cm.

REPLICATES, FISH PER REPLICATE: One replicate, 10 fish per replicate

TEMP PHOTOPERIOD FOOD: Test temperature 22-23 °C, photoperiod 16 hours light and 8 hours dark, food withdrawn one day before exposure,

ANALYTICAL CHEMISTRY DETERMINATIONS: TS measured at one and 96 hours.

Result :

Nominal concentrations were: 50, 100, 1000, 2150, 4640 or 10000 mg/L for test.

Analytical concentrations were: 53, 95, 959, 2146, 4580 or 10221 mg/L at one-hour

Analytical concentrations were: 38, 98, 947, 2084, 4600 or 9935 mg/L at 96-hours

pH measurements at one hour were control to high concentration: 8.6, 8.5, 8.4, 8.5, 8.6, 8.6, 8.6; at 96 hours 8.3, 7.0, 9.8, 8.2, 8.2, nd.

Oxygen levels were above 7 mg/L in most instances at 1, 24, 48, 73, or 96 hours.

Temperature remained at 22° throughout the study.

Mortality: There was no mortality except at the high concentration (10,000 mg/L) where the cumulative mortality at 24 hours was 6/10, at 48 hours was 8/10 at 72 and 96 hours was 10/10.

Clinical signs: The only reported effects were for the 10,000 mg/L group at 24 hours where apathy and tumbling were reported in surviving fish.

Test substance :

2-Pyrrolidone CAS No. 616-45-5 Purity 99.7%

Conclusion :

The 96-hour LC50 is between 4,600 and 10,000 mg/L (based on nominal concentrations). According to the OECD 203 guideline the geometric mean (6,783) of these concentrations may be used to approximate the LC50.
or LC50 = 6,800 mg/L

Reliability : (1) valid without restriction
Guideline study under GLP with no significant problems noted.

Flag : Critical study for SIDS endpoint

13.08.2003 (12)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC0	:	= 500 measured/nominal
EC50	:	> 500 measured/nominal
Limit Test	:	no
Method	:	Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year	:	
GLP	:	no data
Test substance	:	

Method : Daphnia magna (2-24 hours old) were exposed to the test substance in four replicates of five animals (20/group) at nominal concentrations of 0, 31.25, 62.5, 125, 250, or 500 mg/L for 48 hours. The dilution water was prepared from tapwater by dilution with distilled water to reduce the hardness, addition of sulfuric acid to reduce the alkalinity, filtration to remove particulates and passing the water through activated carbon to remove chlorine. Final dilution water had a total hardness of 2.44 mmol/L, an alkalinity of 0.80 mmol/L (to pH 4.3), a calcium:magnesium ratio (molar) of 4:1, a sodium:potassium (molar) ratio of 10:1 and a pH range of 7.7 to 8.3.

Loading of daphnids was 2 ml/daphnid using 10 ml centrifuge tubes. The temperature was maintained at 293 deg K. Diffuse light was on 16 hours/day at an intensity of 570 microSiemens/cm. The dilution water was

bubbled with oil-free air initially to saturate it with oxygen. The test substance dilutions were prepared from a stock at 500mg/l (also the high concentration) by dilution.

Daphnids were examined at 3, 6, 24 and 48 hours after initiation.

Result :

The initial pH did not differ between concentrations and was in the range of 8.11-8.27. The final pH was not concentration dependent and ranged from 7.59 to 8.14. Oxygen concentrations, measure at 0 and 48 hours of the test, were higher at the beginning (9.30-9.42 mg/L) than at the end of the 48 hour exposure period (5.54-8.55) and there was no apparent relationship of DO levels to test-substance concentration.

No daphnids was found immobilized by the treatment and no adverse effects were reported at any concentration.

Test substance :

2-Pyrrolidone CAS No. 616-45-5, distilled, purity > 99.5%

Conclusion :

The NOEC and EC-0 were found to be 500 mg/L
The EC-50 was found to be > 500 mg/L
(These are based on nominal concentrations)

Reliability : (1) valid without restriction
Guideline study, with good documentation including copies of raw data. Although the test did not use analytical measurements of test substance concentration, it is known to be stable in water.

Flag : Critical study for SIDS endpoint
03.08.2003 (8)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 96 hour(s)
Unit : mg/l
EC0 : = 1000 measured/nominal
EC50 : > 1000 measured/nominal
Analytical monitoring : no

Method : Groups of 20 Daphnia magna were exposed to the test substance at either 10, 100, or 1000 mg/L. Groups were made up of four replicates of five daphnids in 300 ml of dilution water containing test substance. Observations were made at least at 24 hours, 96 hours, 7 days, 14 days and 21 days.

Remark :

The stability of the test substance in water was not established. Other information support the test substance being stable in water for at least the initial 48 hour period. Stability at the 3-week time was likely compromised by biodegradation of the test substance.

Result :

No mortality occurred in the first 96 hours of exposure in any group. At the end of the three-week exposure period the number of surviving daphnids was 17/20, 18/20 and 12/20 for the 10, 100 and 1000 mg/L groups, respectively.

Test substance :

2-Pyrrolidone

Conclusion :

The 96-hour EC50 for Daphnia magna is > 1000 mg/L under these conditions.

Reliability : (2) valid with restrictions
Although this study is old and details are limited, the conduct was similar to modern guidelines and the study was conducted according to a scientifically defensible method. The availability of the original data sheets add to the reliability of the work.

31.12.2002 (27)

Type : static
Species : Daphnia pulex (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : = 13.21 calculated
Analytical monitoring : no

Method : Daphnia pulex were cultured in 2-L jars of reconstituted hard water (20OC; pH,7.6-8.0; dissolved oxygen, 60-100% saturation; hardness 160-180 mg/L as CaCO ; alkalinity 110-120 mg/L as CaCO). To minimize leaching, dissolution and sorption of toxicants from the water only glassware and tubing made from perfluorocarbon plastic was used for culturing and testing. The daphnid food was a mixture of the four algal species plus cerophyl at a ratio of 1:1:1:1:4. The daphnids were fed five times a week with 3 mL of food per liter of culture water.

The 48-h tests were conducted with 10 neonates (<24 h old) in five concentrations of each toxicant and the control. Toxicant concentrations (in 150 mL of reconstituted hard water) were at least 50% of the next concentration. The six test beakers, covered with parafilm, were placed in a constant temperature water bath at 20 deg C with a photoperiod of 16 h light, 8 h dark. Test animals were not fed during the experiment. After 48 h the daphnids were pipetted into a watch glass and examined for immobilization.

Mean effective concentration (EC50) and standard error were calculated from the immobilization data for valid toxicity tests (American Society for Testing and Materials 1980). A mean was taken from three valid tests. To calculate EC10, EC50, and EC90 values, we used a computer modification (Peltier et al. 1985) of Finney's (1952) probit analysis. Statistical comparisons were made on logarithmically transformed EC50's using analysis of variance (ANOVA) and Tukey's HSD test (Steel and Torrie 1960).

(Finney DH (1952) Statistical methods in biological assay. C. Griffin and Co Ltd., London, 661 pp)

(Peltier WH, Weber CI(eds) (1985) Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 3rd ed Environ Monitor Support Lab,US Environ Protect Agency, Cincinnati, Rep no 600/4-85-013)

(Steel RGD, Torrie JH (1960) Principles and Procedures of Statistics, McGraw Hill, New York)

Result :

The results from all studies in ther report are presented in the table below:

Compound	EC50 (mg/L) Mean	SE	
DDT (D. magna)		0.0011	0.0001
DDT (17 C)	0.0019	0.0001	
Chlordane (D. magna)	0.097	0.005	
Nicotine	0.242	0.02	
Nicotine (170C)		0.326	0.074
Pentachlorophenol (D. magna)	2.00	0.0	
Pentachlorophenol	2.5	0.1	
1-methylpyrrolidine	2.08	0.20	
Isoxanthopterin		2.97	0.47
2-amino-4,6-dimethylpyridine	9.19	1.85	
2-pyrrolidinone	13.21	4.02	
2-(2-hydroxyethyl)pyridine	13.82	3.60	

Mortality as a function of concentration was not given in the article.

The range of toxicity and the reported SE indicate that studies were conducted in the appropriate concentration range for each test material.

Test substance :

2-Pyrrolidone CAS No. 616-45-5 Purity >= 97%

Reliability : (2) valid with restrictions
Good, this is a published study by a National Laboratory in a peer reviewed journal conducted using a scientifically defensible method. Stability data on the test compound are lacking.

13.08.2003 (25)

Type : static
Species : other aquatic mollusc: Planorbella trivolvis
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 112 measured/nominal
EC0 : = 112 measured/nominal
EC50 : > 112 measured/nominal
Limit Test : yes
Analytical monitoring : no

Method : One group of 10 snails was exposed to a solution of 100 microliters/L test substance at a temperature of 18 C for a period of 96 hours. The initial dissolved oxygen level was 9.2 mg/L and the initial pH was 7.7. The final dissolved oxygen level was 2.6 mg/L with a final pH of 7.2. The snails were identified as Helisoma trivolvis, which are currently known as Planorbella trivolvis.

Result :

All snails survived the 96-hour exposure period.

Test substance :

2-Pyrrolidone

Conclusion :

The 96-hour EC50 for Planorbella trivolvis is > 112 mg/L under these conditions.

Reliability : (2) valid with restrictions
31.12.2002 (27)

Type	:	static
Species	:	other aquatic worm:
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 112 measured/nominal
EC0	:	= 112 measured/nominal
EC50	:	> 112 measured/nominal
Limit Test	:	yes
Analytical monitoring	:	no
Method	:	
Year	:	
GLP	:	no data
Test substance	:	

Method : One group of 10 worms was exposed to a solution of 100 microliters/L test substance at a temperature of 18 C for a period of 96 hours. The initial dissolved oxygen level was 9.2 mg/L and the initial pH was 7.7. The final dissolved oxygen level was 2.6 mg/L with a final pH of 7.2. The aquatic worms were identified as Dugesia tigrine, which is a common freshwater platyhelminth.

Test substance :

2-Pyrrolidone

Conclusion :

The 96-hour EC50 for Dugesia tigrine is > 112 mg/L under these conditions.

Reliability : (2) valid with restrictions

31.12.2002 (1) (27)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC10	:	= 8 calculated
EC50	:	= 84 calculated
Limit test	:	
Analytical monitoring	:	no
Method	:	other: DIN 38412 L9
Year	:	
GLP	:	no
Test substance	:	

Method : Cells were placed in quadruplicate cultures of growth medium according to the method of DIN 38412 L9 containing 0, 25, 50, 100, 250 or 500 mg/L test substance. These concentrations were selected on the basis of a preliminary test at concentrations of 0, 5, 50 or 500 mg/L. Cell counts were determined by counting six replicates from each quadruplicate culture at 0, 24, 48, 72 and 96 hours of incubation. Fluorescence was also determined at these same time-points. pH was measured at the beginning and end of the 90-hour incubation period. The temperature of incubation was a constant 24.8 deg. C.
Statistical Method: Tallerida and Jacob, The Dose-Response Relation in Pharmacology Pages 98-103 pub. Springer Verlag 1979

Remark : the ECOSAR (v0.99f) program using the neutral orgaincs model predicts a 96-hour EC50 of 4777

Result :

The following results are listed in the order
0, 25, 50, 100, 250 or 500 mg/L:
The beginning and end pH values were
Start: 7.84, 7.87, 7.89, 7.86, 7.89, 7.88
End :7.92, 7.99, 8.04, 8.07, 8.12, 8.13

Mean cells counts (X 1000) were:

t= 0: 34, 38, 32, 34, 33, 35
t=24: 106, 94, 88, 62, 51, 51
t=48: 235, 191, 165, 150, 149, 136
t=72: 618, 514, 405, 239, 311, 230
t=96: 1866, 1408, 1042, 334, 279, 407

The changes in fluorescence did not correlate with the cell growth.

From these data the EC10 and EC50 for growth rate at 96 hours were determined to be 20 and 353 mg/L and the EC10 and EC50 for biomass were determined to be 8 and 84 mg/L.

The 72-hour EC10 and EC50 for biomass were 4 and 253 mg/L

Test substance :

2-Pyrrolidone CAS No. 616-45-5, distilled, purity > 99.5%

Reliability : (1) valid without restriction
Guideline study, with good documentation.

Flag : Critical study for SIDS endpoint

31.12.2002 (9)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	aquatic
Species	:	Pseudomonas putida (Bacteria)
Exposure period	:	17 hour(s)
Unit	:	mg/l
EC10	:	= 9268 calculated
Analytical monitoring	:	no
Method	:	other: Bringmann-Kuehn Test
Year	:	1988
GLP	:	no
Test substance	:	

Method : Bacteria were added to flasks containing salts, dilute growth substrate and test material at 0, 156.25, 312.5, 625, 1250, 2500, 5000, 7500, or 10000 mg/L test material. Flasks were incubated for 17 hours at 297 deg K and bacterial growth was estimated by absorption of light at 436 nm.

Remark : At concentrations below 10,000 mg/L, the test substance appears to have stimulated bacterial growth under these conditions.

Result : Bacterial growth, expressed as percent of control after 17 hours incubation was:

TS Conc mg/L	Bacterial growth % of control
0	100
156.25	159
312.5	160
625	162
1250	159
2500	150
5000	151
7500	129
10000	73

Test substance : 2-Pyrrolidone, Distilled
Conclusion : The EC10 was calculated to be 9268 mg/L
Reliability : (2) valid with restrictions
Guideline-type study using a scientifically defensible method.
Documentation good.

08.12.2002 (14)

5.1.1 ACUTE ORAL TOXICITY

Type	:	other: Limit Test
Value	:	> 5000 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	water
Doses	:	5000 mg/kg
Method	:	
Year	:	1979
GLP	:	no data
Test substance	:	

Method : Five rats of each sex were given a single oral dose of test material by oral gavage at a limit dose of 5000 mg/kg-bw. The test material was dissolved in distilled water and administered as a 50% wt/vol solution to Sprague-Dawley rats that had been fasted overnight. Male rats weighed approximately 250 grams and females approximately 200 grams at the time of dosing. Animals were observed regularly for mortality and adverse clinical signs and were weighed on days 4, 7 and 13.

The total observation period before sacrifice was 14 days. Necropsy findings were not given in the report.

Result : No animal died during the study. Average body weights of males were 250, 236, 269 and 297 g on days 0,4, 7 and 13, respectively. Average body weights of females were 200, 201, 211 and 216 g on days 0,4, 7 and 13, respectively. No adverse clinical findings were reported.

Test substance : 2-Pyrrolidone, Pure

Conclusion : The acute oral LD50 of the test substance is greater than 5000 mg/kg bodyweight for both male and female rats.

Reliability : (2) valid with restrictions
Reliability is good as a standard procedure was followed; however, the study lacks details concerning observations and necropsy.

Flag : Critical study for SIDS endpoint
03.08.2003 (5)

Type	:	LD50
Value	:	ca. 8000 mg/kg bw
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	water
Doses	:	
Method	:	
Year	:	1961

GLP	:	no
Test substance	:	
Method	:	The study was conducted as part of the "toxicological pre-testing" for this material. The pre-testing consisted of acute oral dosing of rats, inhalation risk-test in rats, i.p. ALD determination in mice, skin and eye irritation. Details of each procedure are not given in the report.
Result	:	In this study, the ALD50 (Approximate Median Lethal Dose) was stated as about 8.0 g/kg at both 24 hours and 8 days. It is presumed that the observation time was 8 days. Clinical signs were given as convulsions, dyspnea and lying on side; however, it cannot be determined from the report if these signs refer to mice administered TS i.p. or the rats administered TS orally. Likewise, there is no indication of the dose corresponding to these signs or the time of their occurrence.
Test substance	:	2-Pyrrolidone, Distilled, solid

21.11.2002 (13)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	other: Inhalation Risk Test
Value	:	
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	6
Vehicle	:	
Doses	:	
Exposure time	:	8 hour(s)
Method	:	other: BASF Inhalation Risk Test
Year	:	1961
GLP	:	no
Test substance	:	
Method	:	The study was conducted as part of the "toxicological pre-testing" for this material. The pre-testing consisted of acute oral dosing of rats, inhalation risk-test in rats, i.p. ALD determination in mice, skin and eye irritation. Details of each procedure are not given in the report.
Result	:	Under the conditions of this study no animal died as a result of the exposure to saturated vapor for 8 hours. It is noted in the report that no abnormalities were detected at necropsy; however, the length of the post-exposure observation period is not specified in the report.
Test substance	:	2-Pyrrolidone, Distilled, solid
Conclusion	:	It can be concluded that the 8-hour inhalation LD50 for 2-Pyrrolidone is greater than the air saturation concentration of the test substance in air at 30 deg C. Which is approximately 80 ppm.
Reliability	:	(2) valid with restrictions A reliability of 2 is assigned. Although some important details are lacking this study was conducted according to a standard procedure that is scientifically defensible.

21.11.2002 (13)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 2000 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	10
Vehicle	:	
Doses	:	2000
Method	:	OECD Guide-line 402 "Acute dermal Toxicity"
Year	:	1992
GLP	:	yes
Test substance	:	

Method :

Following a quarantine period of at least one week, five healthy male and five healthy female New Zealand Albino rabbits were randomly assigned to the treatment group. The pretest weight range was 2.3 - 2.6 kg for males and 2.1 - 2.5 kg for females. The animals were housed 1/cage in suspended wire mesh cages. Bedding was placed beneath the cages and changed twice/week. Fresh Purina Rabbit Chow (Diet #5321) was provided daily. Water was available ad libitum. The animal room, reserved exclusively for rabbits on acute tests, was temperature controlled, had a 12 hour dark/light cycle.

The test article was used as received and the dose was based on the sample weight as calculated from the specific gravity. The test article was applied to the prepared dermal site, one time, by syringe type applicator at a dose level of 2.0 g/kg. The test site was covered with a gauze patch, secured with non-irritating tape and gentle pressure was applied to the gauze to aid the distribution of the test article over the area. The torso was wrapped with plastic that was secured with non-irritating tape. At 24-hours after initiation, the patches were removed and residual test article was removed with distilled water.
.
The animals were observed 1, 2 and 4 hours post dose and once daily for 14 days for toxicity and pharmacological effects. Animals were observed twice daily for 14 days for mortality. The test sites were scored for dermal irritation at 24 hours post dose and on days 7 and 14 using the numerical Draize scale
Body weights were recorded pretest, weekly and at death or termination. All animals were examined for gross pathology. Abnormal tissues were preserved in 10% buffered formalin and saved for possible future microscopic examination.

Result :

All animals survived the 2000 mg/kg dermal application. There were no abnormal systemic signs noted in 9/10 animals. One male exhibited red staining of the nose/mouth area and an apparent cataract in the right eye on day 5, with the ocular abnormality persisting through day 14 but this was considered to result from a slef-inflicted injury unrelated to test material administration. Body weight gains were normal at all weighing periods. Dermal reactions were slight to well-defined on day 1 but were absent on days 7 and 14. Necropsy did not reveal any treatment related changes.

Test substance	:	2-Pyrol, no further information
Conclusion	:	The dermal LD50 was found to be > 2000 mg/kg-bw
Reliability	:	(1) valid without restriction

Guideline study under GLP with no significant problems noted.

Flag : Critical study for SIDS endpoint

30.11.2002 (24)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	drinking water
Exposure period	:	90 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	600, 2400, 7200 or 15000 ppm in drinking water
Control group	:	yes, concurrent vehicle
NOAEL	:	= 2400 ppm
LOAEL	:	= 7200 ppm
Method	:	OECD Guide-line 408 "Subchronic Oral Toxicity - Rodent: 90-day Study"
Year	:	1981
GLP	:	yes
Test substance	:	

Method : 2-Pyrrolidone was administered to groups of 10 male and 10 female Wistar rats at doses of 0; 600; 2,400; 7,200 and 15,000 ppm in the drinking water over a period of 3 months.

Wistar rats (Chbb: THOM (SPF)) were obtained from Dr. Karl Thomae GmbH, Biberach/Riss, FRG. Rats were identified unambiguously by ear tattoo. Animals were individually housed in type DK III stainless steel wire cages Becker & Co., Castrop-Rauxel). Animal rooms were air-conditioned with temperatures in the range 20 - 24°C and relative humidity in the range 30 - 70%. The day/night cycle was 12 hours (light from 06.00 a.m. - 06.00 p.m.).

Test solutions were analysed at the start and end of the study to assure that the concentrations were correct and the 4-day stability was assessed as 97%. The mixtures were prepared at no less than 4-day intervals. Water consumption was determined once/week over a period of 4-days. Animals were weighed weekly and given a thorough physical examination at each weighing. Food consumption was determined weekly. Urine samples were taken on day 85, blood was sampled and analyzed on study day 88, the final bodyweight was recorded on day 91 and necropsies were conducted over days 92 to day 95,

Food consumption, water consumption and body weight were determined each week. The animals' state of health was checked each day. When the animals were weighed they were subjected to an additional comprehensive clinical examination

Clinincal chemistry parameters were: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase - serum-gamma-glutamyltransferase

Blood chemistry parameters were: sodium, potassium, chloride, inorganic

phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, magnesium.

In addition complete hematology and urinalysis were conducted.

At necropsy, major organs were weighed and sections were fixed for histopathology. All animals were subjected to gross-pathological assessment, followed by histopathological examination using a complete tissue list.

Statistical methods: Means and standard deviations for the variables food consumption, body weight, body weight change, water consumption and test substance intake (except control group) were calculated for the animals of each test group. They were printed out in the summary and individual value tables, with the exception that for test substance intake and body weight change only summary tables were prepared. For the parameters food consumption, water consumption, body weight and body weight change a parametric one-way analysis of variance was done via the F-test (ANOVA). If the resulting p-values were equal to or less than 0.05, a comparison of each dose group with the control group was carried out. These comparisons were performed simultaneously via Dinnett's test for the hypothesis of equal means. If the results of this test were significant, labels (* for,$p < 0.05$, ** for $p < 0.01$) were printed together with the group means in the tables. Both tests were performed two-sided. Statistical analysis of histopathology was conduced with a proprietary computer program.

The following tissues were examined and preserved at necropsy:
- brain
- pituitary gland
- thyroid and parathyroid glands
- thymus
- trachea
- lungs
- heart
- aorta
- salivary glands (mandibular and sublingual)
- liver
- spleen
- kidneys
- adrenal glands
- pancreas
- testes/ovaries
- uterus/vagina
- epididymides, prostate, seminal vesicles
- skin
- esophagus
- stomach (forestomach and glandular stomach)
- duodenum
- jejunum
- ileum
- cecum
- colon
- rectum
- urinary bladder
- lymph nodes (mesenteric, mandibular)

- female mammary gland
- skeletal muscle
- sciatic nerve
- bone marrow (femur)
- eyes
- femur with knee joint
- sternum with marrow
- spinal cord (cervical, thoracic and lumbar)
- extraorbital lacrimal gland
- all gross lesions

The Following Tissues Were examined microscopically in high-dose and control animals (and other groups as indicated)

- brain
- pituitary gland
- thyroid
- parathyroid
- thymus (and all females in all groups)
- trachea
- lungs (and all animals in all groups)
- heart
- aorta
- salivary glands (mandibular and sublingual)
- liver (and all animals in all groups)
- spleen
- kidneys (and all animals in all groups)
- adrenal glands (and all animals in all groups)
- pancreas
- testes/ovaries
- uterus/vagina
- epididymides, prostate, seminal vesicles
- skin
- esophagus
- forestomach (and all animals in all groups)
- glandular stomach (and all animals in all groups)
- duodenum
- jejunum
- ileum
- cecum
- colon
- rectum
- urinary bladder
- lymph nodes (mesenteric, mandibular)
- female mammary gland
- skeletal muscle
- sciatic nerve
- sternum with marrow
- femur with joint
- bone marrow (femur)
- eyes
- femur with knee joint
- sternum with marrow
- spinal cord (cervical, thoracic and lumbar)
- All gross lesions were examined in all groups

Remark :

For a full description of the procedures used to examine reproductive organs see the robust summary for fertility.

The study was carried out according to following guidelines:

- EC Commission Directive 87/302/EEC of 18 November, 1987; Part B: Methods for the determination of Toxicity; Sub-chronic Oral Toxicity Test; 90-day repeated oral dose using rodent species; Official Journal of the European Communities No. L 133, p. 8-11, 1988

- OECD Guidelines for Testing of Chemicals; Method No. 408: Subchronic Oral Toxicity - Rodent: 90-day study; May 12, 1981

Result :

Substance intake::
Mean test material consumption in mg/kg- day were:
+ males: 33, 184, 529 and 1062 mg/kg
+ females 42, 230, 643 and 1189 mg/kg

No animal died during the study and no adverse clinical signs were noted.

Other effects by dose group:

*** Test group 4 (15,000 ppm; about 1,125 mg/kg body weight)
-decreased food and water consumption in both sexes
- decreased body weight gains, male's BW were 9% lower than controls and female's were 8% lower than controls on day 91
- prolonged prothrombin times in rats of each sex
- decrease in total protein, globulins, triglycerides and creatinine in both sexes
- increased urinary specific gravity in the males - reduced urinary volume in the males
- dark yellow discoloration of urine specimens in the males
- increase in the mean relative kidney weights in males and females

*** Test group 3 (7,200 ppm; about 586 mg/kg body weight)
- slight decrease of food consumption in female animals
- slight decrease of water consumption in both sexes
- slightly decreased body weights in females, 6% less than controls on day 91
- decreased body weight gains of 7% (males) and 16% (females) on day 91
- decrease in creatinine in both sexes
- decrease in total protein in the females
- increased urinary specific gravity in the males - reduced urinary volume in the males
- dark yellow discoloration of urine specimens in the males
- increase in the mean relative kidney weights in males

*** Test group 2 (2,400 ppm; about 207 mg/kg body weight) and
- no substance-related effects

*** Test group 1 (600 ppm; about 37 mg/kg body weight)
- no substance-related effects

Mean Terminal Body and Kidney Weights (Absolute and Relative)

MALES (grams)

Group	Body	Kidney (absolute)	Kidney (relative)
0	471	2.97	0.68
600	460	2.93	0.69
2400	458	3.05	0.72
7200	452	3.11	0.73*
15000	428*	3.13	0.77**

FEMALES (grams)

Group	Body	Kidney (absolute)	Kidney (relative)
0	265	1.92	0.79
600	263	2.00	0.83
2400	269	1.99	0.80
7200	248	1.93	0.84
15000	242*	2.03	0.89**

Note: A finding of "altered cellular composition of the thymic cortex" was reported in all dosed groups of females. A second 90-day study was conducted at 0, 50 and 15,000 ppm in drinking water using groups of five female rats to investigate the significance of this finding. It this second study the identical finding was present; however, it also occurred in controls. In addition, retrieval and examination of thymus slides from controls animals in other studies were examined and were also found to have the same "pathology". Therefore, this was considered incidental and not compound related.

Test substance :

2-Pyrrolidone CAS No. 616-45-5 Purity 99.7%

Conclusion :

The kidney appears to be a target organ at dose levels of 7,200 ppm (about 586 mg/kg) in the drinking water and above. The NOAEL is 2,400 ppm in drinking water or about 207 mg/kg-bw-day

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

03.08.2003 (10)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Salmonella typhimurium reverse mutation assay

System of testing :

Test concentration : 0, 0.1, 1.0, 5.0, 10, 25, 50, 100 and 150 microliters per plate

Cycotoxic concentr. : 150 microliters per plate

Metabolic activation : with and without

Result : negative

Method : other

Year : 1987

GLP : yes

Test substance :

Method : S. typhimurium strains TA1535, TA1538, TA100, TA1537, TA98 were tested using a plate incorporation technique both with and without metabolic activation. Aroclor 1254 induced rat liver S-9 was used for metabolic activation at a rate of 0.5 ml S-9 per plate when used with the overlay procedure. Test and control materials were incorporated directly into the overlay agar with the bacteria.

Plates were prepared and read in triplicate and the entire assay was repeated a second time (independent repeat). Colonies were counted using an automated Biotran II colony counter except when accurate counts could not be obtained (e.g. precipitate formation) .

Concentrations of test substance were selected based on a preliminary toxicity assay at 14 concentration levels using two-fold dilutions from a high concentration of 150 microliter per plate (for liquids). 150 microliters per plate was used as the top concentration in the studies because this is the limit dose for the test and because this concentration reduced the number of TA-100 revertant colonies by approximately 50% in a preliminary dose-rangefinding test.

Concentrations tested were 0, 0.1, 1.0, 5.0, 10, 25, 50, 100 and 150 microliters per plate for all strains in both of the two independent repeats.

The solvent and negative control substance was distilled water. Positive controls were:
Without metabolic activation
Sodium azide at 10 mcg/ plate for strain TA-1535 and TA-100
Quinacrine mustard at 5 mcg/ plate for strain TA-1537
2-Nitrofluorene at 10 mcg/ plate for strains TA-1538 and TA-98

With metabolic activation,
2-Anthramine at 2.5 mcg/ plate for all strains

Statistical Methods

Formal statistical methods were not used to evaluate the data. Evaluations considered if a dose-response was observed and strain-specific evaluation criteria.

For strains TA-1535, TA-1537 and TA-1538, the data set is evaluated as positive if a dose-response is observed over a minimum of three test concentrations and the increase in revertants is equal to or greater than three times the solvent control value at the peak of the dose-response. The solvent control value should be within the normal range for evaluating the results.

For strains TA-98 and TA-l00, the data set is evaluated as positive if a dose-response is observed over a minimum of three test concentrations and the increase in revertants achieves a doubling of the solvent control value at the peak of the dose-response. The solvent control value should be within the normal range for evaluating the results.

Result : In the preliminary study on TA-100, the test material was toxic to the indicator only at 150 microliters per plate as evidenced by the reduced number of revertants on the minimal media plates (about a 50% reduction).

The results of the initial and independent assays conducted on the test material at dose levels ranging from 0.1 to 150 microliters per plate in the

absence and presence of metabolic activation did not exhibit increased numbers of his+ revertant colonies.

The positive control treatments in both the nonactivation and S9 activation assays induced large increases in the revertant numbers with all the indicator strains, which demonstrated the effectiveness of the S9 activation system and the ability of the test system to detect known mutagens.

Test substance : 2-Pyrrolidone CAS No. 616-45-5 Purity by GLC 99.9 Area % source BASF

Conclusion : The test material, 2-Pyrrolidone, did not exhibit genetic activity in any of the assays conducted in this evaluation and was not mutagenic to the Salmonella tvphimurium indicator organisms under the test conditions according to the established evaluation criteria.

Reliability : (1) valid without restriction
Guideline-like study under GLP

Flag : Critical study for SIDS endpoint

06.08.2003 (22)

Type : other: Aneuploidy Induction in Yeast
System of testing : Saccharomyces cerevisiae
Test concentration : 0, 289.6, 321.0, 352.2, 383.3, 414.2, or 445.0 mM
Cycotoxic concentr. : 321 and above
Metabolic activation : without
Result : positive
Method :
Year : 1987
GLP : no data
Test substance :

Method : Diploid strain D61.M of Saccharomyces cerevisiae, developed by F.K. Zimmermann, was used for the detection of aneuploidy and other genetic events. Its genetic constitution and the detailed procedures for its use in detecting aneuploidy have been previously described in detail. In brief: recessive alleles (cyh2, cycloheximide resistance; ade6, white-adenine requirement; leul, leucine requirement) of three genes are arranged on both sides of the centromere on one copy of chromosome VII. Simultaneous expression of all three recessive alleles in the same clone can result either from loss of the homologous chromosome VII carrying the wild-type alleles or from simultaneous multiple events of recombination or mutation, which are expected to be extremely rare.

Ten parallel 5-ml cultures were grown in YEPD medium until they attained a titer of approximately 5-7 x 10exp7 cells/ml. A 0.1-ml aliquot was removed from each culture and plated onto the cycloheximide-YEPD medium to select cultures with low spontaneous rates of cycloheximide resistance. The 5-ml cultures were stored at 4°C until use. A culture that was determined to have a low spontaneous frequency of cycloheximide resistance (typically < 1 x 10exp6) was diluted 1:10 into fresh YEPD medium and incubated at 28C for 4 hr to bring the cells into exponential growth phase before addition of the test chemical.
The exponential phase culture was adjusted to 5 x 10exp6 cells/ml in YEPD medium. Treatments were carried out in 2-nil aliquots in glass test tubes by adding microliter quantities of the test chemical either directly or from a stock solution of the chemical in water prepared just before use. The concentration of the stock solutions was dictated by the level of toxicity, which had been determined in preliminary experiments. The growing yeast

cells were treated in a shaker water bath at 28°C for 4 hr; then the cultures were refrigerated at 4°C in a water bath for 16 hr. The cold holding period was followed by a second 4-hr incubation at 28°C before the cultures were diluted and plated on the appropriate media. (The interruption of growth by cold temperature storage greatly enhances the induction of aneuploidy by a number of solvent chemicals). When necessary, cultures were diluted to approximately 1-2 x 10exp7 cells/ml, and 0.1-ml aliquots were plated directly onto the selective cycloheximideYEPD medium to determine the resistant population. Appropriate dilutions were plated onto YLPD medium to determine the surviving population. Plates were incubated for 5-7 days, and colonies were enumerated. On selective cycloheximide-YEPD medium the resistant colonies were either red or white. The red colonies resulted from the occurrence of genetic events such as gene conversion or mutation affecting the CYH2 locus only and not from chromosome malsegregation. The cycloheximide-resistant white colonies are presumably due to chromosome loss because the recessive cyh2 and the recessive ade6 alleles are being simultaneously expressed. To confirm that the white resistant colonies are really monosomic for chromosome VII, each colony to be tested was streaked onto YEPD master plates, which were incubated overnight at 28C, and then replicas were plated onto both a synthetic complete medium and onto the same medium lacking leucine. White (ade6) and cycloheximide-resistant (cyh2) colonies must also require leucine (leu1) to be considered monosomic.

Remark : In a subsequent paper, these same authors found no aneuploidy potentiation of 2-Pyrrolidinone with nocodazole. They discussed the potential mechanism of solvent-induced aneuploidy in terms of the fact that microtubles dissociate in the cold to their tubulin subunits and polymerize again as the temperature is raised. The solvents were speculated to inhibit or accelerate the rate of repolymerization (Mayer and Goin, Mut Rech. 201:413-421, 1988).
Several factors indicate that this result is not relevant to hazard assessment to man.

Solvent-induced aneuploidy appears to be a special case.

Solvent-induced aneuploidy is enhanced by cold incubation, which was part of the protocol in this investigation.

The concentration range where effects are reported is narrow range and coincides with toxicity.

The concentrations where effects are reported are extremely high and impossible to achieve under normal industrial conditions in man.

Common non-genotoxic solvents such as acetone are known to induce this effect under the special conditions employed in this study.

Result : Positive results on the induction of aneuploidy by 1-methyl-2-pyrrolidirone and 2-pyrrolidinone were recorded as the number of cycloheximide-resistant white colonies observed and the fraction of these colonies that were Leu-. Aneuploidy frequencies were calculated by using these numbers as the numerator and the population screened as the denominator. In cases in which only a few white colonies were found, all were tested for their leucine requirement. When many white colonies were observed, all were counted, and a representative number (usually 25) was tested. The number of red cycloheximide-resistant colonies was

determined and was found not to increase with test material concentration. As red-resistant colonies arise as a result of other genetic events, they served as a control showing that other genetic effects such as mutation or recombination were not induced by the test chemical.

The frequency of aneuploidy increased with the dosage of each test chemical. 1-Methyl-2-pyrrolidinone was active between 150 and 230 mM, while 2-pyrrolidinone was active between 350 and 450 mM, and appeared to be slightly less toxic in comparable ranges. As there was no increase with concentration for either chemical in the frequency of the red cycloheximide-resistant colonies. Therefore, aneuploidy rather than other nuclear genetic effects were being induced by these chemicals.

Data are shown in the table.

Test substance : 2-Pyrrolidone CAS No. 616-45-5 from Aldrich Chemical Co
Attached document : Y-table-HP600.bmp

Test M Conc (mM)	Percent Survival	Pop Screened X 10^6	Total White Colonies	Aneuploidy Frequency x10^6 CFU
0	100	4.73	10	1.27
289.6	98	5.20	42	6.79
321.0	61	4.35	48	9.71
352.2	42	4.43	65	10.56
383.3	23	3.28	98	17.93
414.2	8	1.75	120	21.94
445.0	7	1.50	120	19.20

Reliability : (2) valid with restrictions
The method was well described and sufficient details and data were presented to indicate that this study has good reliability.

28.11.2002 (23)

Type : Cytogenetic assay
System of testing :
Test concentration :
Cycotoxic concentr. : High doses minimally cytotoxic.
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 473
Year : 1987
GLP : yes
Test substance :

Method : 2-Pyrrolidone was tested for its ability to induce chromosomal aberrations in human lymphocytes following in vitro exposure in the presence and absence of a metabolizing system.
Based on a pretest to determine the highest experimental dose and in consideration of the cytotoxicity actually found in the present cytogenetic investigations, 3500 mcg/ml, 2500 mcg/ml and 1250 mcg/ml culture medium in the experiment without S-9 mix. or 6000 mcg/ml, 5000 mcg/ml and 2500 mcg/ml culture medium in the experiment with metabolic activation, were selected. This selection was based on the quality of the metaphases and not on the mitotic index because the test substance

concentrations causing reduction in the mitotic index are at dose levels that severely affect chromosomes; thus, no longer allowing evaluation.

Duplicate cultures were used for all experimental points. The solvent was distilled water.

Negative controls (untreated and solvent) and positive controls both without S-9 mix (0.2 mcg mitomycin C/ml culture medium) and with metabolic activation (6 mcg cyclophosphamide/ml culture medium) were also tested.

Heparinized human venous blood was added to the culture medium (chromosome medium 1A with PHA). After mitogen stimulation of the lymphocytes using PHA and incubation at 37°C for 48 hours. The cultures were treated with test substance without S-9 mix for 24 hours; in the experiment with S-9 mix (from Aroclor-induced rats) test substance treatment lasted 2 hours followed by a reincubation for 22 hours using fresh culture medium without test substance. About 2 - 3 hours prior to harvesting the cells, Colcemid was added to arrest cells in a metaphase-like stage of mitosis (C-metaphase). After preparation of the lymphocyte chromosomes and staining with Giemsa, 100 metaphases of each culture in the case of the test substance, untreated control and solvent control, or 50 cells of each culture in the case of positive controls, were analyzed for chromosomal aberrations.

Statistical Procedure:

The Fisher exact test was applied to determine significant differences between the relative frequencies of a characteristic of two groups, and it was used to answer the questions of whether there are significant differences between control groups (untreated controls and solvent controls) and dose groups with regard to the rate of structural aberrant metaphases.

Result **:** ** Assay without metabolic activation:::
Untreated controls
10 (5.0%) aberrant cells including gaps and 2 (1,0%) aberrant cells excluding gaps were found

Solvent controls:
12 (6.0%) aberrant metaphases including gaps and 5 (2.5%)aberrant metaphases excluding gaps were found

3500 mcg/ml:
8 (4.0%) chromosomally damaged cells including gaps and 2 (1.0%) aberrant cells excluding gaps were detected.

2500 mcg/L:
14 (7.0%) aberrant metaphases including gaps and 6 (3.0%) chromosomally damaged cells excluding gaps were observed.

1250 mcg/ml:
17 (8.5%) aberrant cells including gaps and 2 (1.0%) aberrant metaphases excluding gaps were found.

0.2 mcg mitomycin C/ml:
With 44 (44%) aberrant cells including gaps and 37 (37%) aberrant mitosis excluding gaps including 2 multiple aberrant metaphases and 5 cells with

exchanges, the positive control substance led to the expected increase in the number of chromosomally damaged cells.

No differences regarding aneuploidies (hyperploid metaphases) and polyploidies between the various dose groups and the negative controls were observed.

Assay with metabolic activation:::

Untreated control:
4 (2.0%) aberrant mitosis including gaps only were found.

Solvent contro1:
15 (7.5%) aberrant metaphases including gaps and 4 (2.0%) chromosomally damaged cells excluding gaps were found.

6000 mcg/ml:
17 (8.5%) chromosomally damaged cells including gaps and 2 (1.0%) aberrant cells excluding gaps were observed.

5000 mcg/ml:
16 (8.0%) chromosomally damaged cells including gaps and 1 (0.5%) aberrant cells excluding gaps were observed.

2500 mcg/ml:
13 (6.5%) chromosomally damaged cells including gaps and 1 (0.5%) aberrant cells excluding gaps were observed.

6 mcg cyclophosphamide/ml:
27 (27%) chromosomally damaged cells including gaps and 20 (20%) aberrant cells excluding gaps were observed, which was the expected increase for positive controls.

No differences regarding aneuploidies (hyperploid metaphases) and polyploidies between the various dose groups and the negative controls were observed.

Test substance : 2-Pyrrolidone CAS No. 616-45-5 Purity 99.9%

Conclusion : According to the results of the present study, the test substance 2-pyrrolidone did not lead to any increase in the number of aberrant metaphases including and excluding gaps when compared to the solvent controls either without S-9 mix or after adding a metabolizing system. 2-Pyrrolidone is evaluated not to be a chromosome-damaging (clastogenic) agent under in vitro conditions using human lymphocytes, under these experimental conditions.

Reliability : (1) valid without restriction
Guideline study under GLP with no significant problems noted.

Flag : Critical study for SIDS endpoint
29.11.2002 (2)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female
Strain	:	NMRI
Route of admin.	:	i.p.
Exposure period	:	16, 24 and 48 hours
Doses	:	2000, 1000, and 500 mg/kg-bw
Result	:	negative
Method	:	OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year	:	1993
GLP	:	yes
Test substance	:	

Method :

Male and female animals (NMRI mice, Charles River GmbH, WIGA) were assigned to the test groups using a randomization plan prepared with an appropriate computer program. Animals were housed in Makrolon cages, in groups of 5 according to sex in fully air-conditioned rooms with a range of 20 - 24°C for temperature and a range of 30 - 70% for relative humidity. Before treatment, animals were transferred to Makrolon cages and housed individually under the same conditions until the end of the test. The day/night rhythm was 12 hours (light from 6.00 - 18.00 hours). Standardized pelleted feed (Kliba Haltungsdidt, Klingentalmühle AG) and drinking water from bottles were available ad libitum.

Doses selected were 2000, 1000 and 500 mg/kg-bw and were selected on the basis of a preliminary toxicity study. In this study, the highest recommended dose of 2000 mg/kg was administered and survived by all animals but led to signs of toxicity such as irregular respiration, piloerection, abdominal position, apathy and squatting posture; the general state of the animals was poor.

Five Male and female animals per sacrifice interval and dose group were given test substance dissolved in distilled water 2000 mg/kg, 1000 mg/kg and 500 mg/kg body weight. Treatment consisted of a single intraperitoneal administration with a volume of 10 ml/kg body weight. As a positive control, 20 mg of cyclophosphamide/kg body weight or 0.15 mg of vincristine/kg body weight, both dissolved in distilled water, were administered to groups (five animals total, either 2 or 3 of each sex) of male and female animals once intraperitoneally each in a volume of 10 ml/kg body weight. All test substance formulations were prepared immediately before administration.

Sacrifice intervals per dose-group were:

2000 mg/kg;	16, 24 and 48 hours
1000 mg/kg;	24 hours
500 mg/kg	24 hours
Controls	24 hours

Preparation of bone marrow: After cutting off the epiphyses, the bone marrow was flushed out of the diaphysis into a centrifuge tube using a cannula filled with fetal calf serum which was at 37°C (about 2 ml/femur). The suspension was mixed thoroughly with a pipette, centrifuged at 1500 rpm for 5 minutes, the supernatant removed the cells were resuspended. One drop of this suspension was dropped onto clean microscopic slides. Smears were prepared using slides with ground edges, the preparations were dried in the air and subsequently stained in eosin and methylene blue solution for 5 minutes, rinsed, placed in fresh distilled water for 2 or 3 minutes and finally stained in Giemsa solution for 12 minutes. After being rinsed twice and clarified with xylene, the preparations were embedded in Corbit-Balsam. Slides were coded before microscopic analysis.

Evlauations: In general, 1000 polychromatic erythrocytes from each male and female animal of every test group was evaluated and investigated for micronuclei. The normochromatic erythrocytes which occur were also scored. The following parameters were recorded:
Number of polychromatic erythrocytes
Number of polychromatic erythrocytes containing micronuclei
Number of normochromatic erythrocytes
Number of normochromatic erythrocytes containing micronuclei
Ratio of polychromatic to normochromatic erythrocytes
Number of small micronuclei (d < D/4) and of large micronuclei (d >_ D/4)

No statistical methods were employed in data analysis.

Result :

Clinical examinations: The single intraperitoneal administration of the solvent in a volume of 10 ml/kg body weight was tolerated by all animals without any signs or symptoms. A dose of 2000 mg/kg body weight of test substance, led to irregular respiration, piloerection, abdominal position and apathy about 30 minutes after administration; the general state of some animals was poor. After treatment of the animals with 1000 or 500 mg/kg, only irregular respiration and piloerection were observed after about 30 minutes. After about 1 - 2 hours clinical signs were no longer observed. Neither the single administration of the positive control substance cyclophosphamide in a dose of 20 mg/kg-bw nor that of vincristine at 0.15 mg/kg-bw caused any evident signs of toxicity.

Micronulei: Mean polychromatic erythrocytes containing micronuclei were:

Negative control (24 hrs)	1.5%
2000 mg/kg (16 hrs)	1.2%
2000 mg/kg (24 hrs)	1.7%
2000 mg/kg (48 hrs)	1.6%
1000 mg/kg (24 hrs)	2.4%
2000 mg/kg (16 hrs)	1.2%
Cyclophosphamide (24 hrs)	13.6%
Vincristine (24 hrs)	83.2%

Administration of test substance did not lead to any increase in the rate of micronuclei. The number of normochromatic or polychromatic erythrocytes containing small micronuclei (d < D/4) or large micronuclei (d > D/4) did not deviate from the solvent control value at anv sacrifice interval. No inhibition of erythropoiesis induced by the treatment of mice with Pyrrolidon-2 was detected; the ratio of polychromatic to normochromatic erythrocytes was always in the same range as that of the control values in all dose groups.

The number of normochromatic erythrocytes containing micronuclei did not differ to any appreciable extent in the negative control or in the various dose groups at any of the sacrifice intervals.

Test substance : 2-Pyrrolidone CAS No. 616-45-5 Purity > 99.5%

Conclusion :

The number of normochromatic erythrocytes containing micronuclei did not differ to any appreciable extent in the negative control or in the various dose groups at any of the sacrifice intervals.

Reliability : (1) valid without restriction
Guideline study under GLP with no significant problems noted.

Flag : Critical study for SIDS endpoint

29.11.2002 (4)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : other: Reproductive Organ Examination from 90-Day Study
Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : drinking water
Exposure period : 90-days
Frequency of treatm. : daily
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 600, 2400, 7200 or 15000 ppm in drinking water
Control group : yes, concurrent vehicle

Method : 2-Pyrrolidone was tested for subchronic toxicity in a 90-day study. The test substance was administered in drinking water to groups of 10 Wistar rats (Strain Chbb:THOM (SPF)) 10 of each sex - in dose groups of 15000, 7200, 2400, 600 and 0 ppm.

Methods followed the European and international guidelines:
EC Commission Directive 87/302/EEC of November 18, 1987; Part B: Methods for the determination of Toxicity Sub-chronic Oral Toxicity Test, 90-day repeated oral dose using rodent species; Official Journal of the European Communities No. L 133, pages 8-11, 1988; and OECD Guideline for Testing of Chemicals; Method No. 408: Subchronic Oral Toxicity - Rodent: 90-day study; May 12, 1981.

For a more detailed description of the 90-day study conduct and results please see the robust summary for the study in the repeated-dose section.

Briefly: Test solutions were analysis at the start and end of the study to assure that the concentrations were correct and the 4-day stability was assessed as 97%. The mixtures were prepared at no less than 4-day intervals. Water consumption was determined once/week over a period of 4-days. Animals were weighed weekly and given a thorough physical

examination at each weighing. Food consumption was determined weekly. Urine samples were taken on day 85, blood was sampled and analyzed on study day 88, the final bodyweight was recovered on day 91 and necropsies were conducted over days 92 to day 95.

This robust summary will describe the methods and results of reproductive organ evaluation.

Organ Weights
The testes were weighed in all male rats, and the ovaries were weighed in all female rats. Absolute weights as well as relative weights (related to the terminal body weight) were determined and assessed statistically, using Dunnett's test (two-sided).

Gross lesions
During necropsy, specific attention was given to gross lesions of male reproductive organs (testes, epididymides, prostate gland, seminal vesicles and coagulating glands) and female reproductive organs (ovaries, including oviducts, uterus, including cervix uteri and vagina). In addition, the adrenal glands of all animals were inspected grossly during necropsy and after appropriate fixation; the pituitary glands of all animals were assessed grossly during necropsy after removal of the brain as well as during removal from the skull after appropriate fixation. Further, in females, special attention was given to the gross appearance of the mammary gland and the external genitalia (males: penis, preputium, scrotum, processus vaginalis; females: vulva) were also inspected carefully during necropsy.

Histopathology
The reproductive organs of male rats (testes, epididymides, prostate gland, seminal vesicles and coagulating glands) and the reproductive organs of female rats (uterus, including cervix uteri, ovaries, including oviducts and vagina) were fixed routinely in a 4% aqueous solution of formaldehyde for at least 48 hours. In addition, the adrenal glands and the pituitary gland (both sexes) and parts of the mammary gland (female rats) of all animals were fixed in formaldehyde solution. Any gross lesions noted during necropsy in the external or internal sex organs of male or female rats were also fixed in 4% aqueous formaldehyde solution.

After fixation, the reproductive organs of male (both testes, both epididymides - comprising caput, corpus and cauda epididymidis, prostate gland - comprising dorsolateral and ventral parts, seminal vesicles with attached coagulating glands) and of female rats (both ovaries, uterus, including cervix uteri and vagina) as well as the pituitary gland and the female mammary gland were trimmed, processed to paraplast blocks, cut at a thickness of approximately 3 microns and stained with hematoxylin and eosin (H.& E.). The slides of all animals of the control and of the high dose group were assessed using a light microscope with primary magnifications between 25-400 x. Adrenal glands of all animals were processed, stained with H.& E. and assessed histopathologically.

During histopathological evaluation of reproductive organs, the following were specifically considered:

Testes: histopathology was performed on mid cross sections through both testes. Besides gross lesions such as atrophy or tumors, testicular

histopathological examination looked for treatment-related effects such as focal or diffuse atrophy of the seminal epithelium, retained spermatids, missing germ cell layers or types, multinucleated giant cells or sloughing of spermatogenic cells into the tubular lumen. In addition, attention was given to the morphology of the Sertoli cells (vacuolization) and to the interstitial cells of Leydig (number and morphology).

Epididymides: the examination was performed on a mid longitudinal section through both epididymides, comprising caput, corpus and cauda epididymidis. Besides gross lesions such as atrophy, special attention was given to the presence of sperm granulomas, leukocytic infiltration (inflammation), aberrant cell types within the lumen, and oligospermia or aspermia.

Prostate Gland: histopathology was performed on cross sections through the dorso-lateral and ventral parts of the gland. Special attention was given to looking for inflammatory reactions (acute or chronic, purulent, mixed cellular or lymphocytic, in the glandular acini or in the interstitium). Moreover, the morphology of the acinar cells was assessed carefully (hypertrophy, hyperplasia, atrophy) as was the functional status of the gland (amount of colloid in the acini and its staining properties).

Seminal vesicles: both glands were investigated using cross sections through the mid part of the gland. The attached coagulating glands were also examined (although they were not protocol organs and were, hence, not separately mentioned in the tables of the report). Special attention was given to looking for findings of inflammatory reactions (acute or chronic, purulent, mixed cellular or lymphocytic, in the glandular acini or in the interstitium). Moreover, the morphology of the acinar cells was assessed carefully (hypertrophy, hyperplasia, atrophy) as well as the functional status of both glands (amount of fluid in the acini and its staining properties).
Ovaries: histopathological examination was performed on mid cross sections through both organs. Assessment was focused on possible detection of qualitative depletion of the primordial and growing follicle populations, as well as the presence/absence of antral follicles (Graafian follicles) and corpora lutea. Special attention was given to the ovarian interstitium and its cell populations with regard to, atrophy, hypertrophy and/or hyperplasia. A differential ovarian follicle count (DOFC, to detect a quantitative depletion of primordial and/or growing follicles) was not performed.

Oviducts: Not investigated histopathologically.

Uterus (including cervix uteri, which was not listed separately): histopathology was performed on cross sections through the mid part of each uterus horn and on a mid longitudinal section through the cervix uteri with the portio on one side and the base of the uterine horns on the other side. Special attention was give to looking for findings of inflammatory reactions (acute or chronic, purulent, mixed cellular or lymphocytic, in the mucosa or in the glands). Moreover, the morphology of the epithelium, the glands and the musculature were assessed carefully (e.g. for hypertrophy, hyperplasia, atrophy). No specific consideration was given to the status of the sexual cycle according to the cellularity in uterus and cervix uteri.

Vagina: a longitudinal section was performed. Major possible findings which were investigated were inflammatory reactions (acute or chronic, purulent, mixed cellular or lymphocytic in the lumen and/or the wall).

Moreover, the morphology of the epithelium and the underlying musculature was assessed carefully (e.g. for hypertrophy, hyperplasia, atrophy). No specific consideration was given to the status of the sexual cycle according to the cellularity in the vagina.

Remark :

Comparison of methodology to pathology performed in a reproductive study:

With the exception of the oviducts, all organs of the male and female genital tract that are examined in a "modern" reproduction toxicity study (e.g. US-EPA OPPTS 870.3800) were investigated both grossly and histopathologically. Further, all other organs required for histopathology in the OPPTS 870.3800 guideline - namely the pituitary and the adrenal glands - were investigated histopathologically. The only significant deviation from OPPTS 870.3800 was that uterus (with oviducts and cervix), epididymides (total weights for both and cauda weight for either one or both), seminal vesicles (with coagulating glands and their fluids), prostate gland, pituitary gland and spleen were not weighed. These organs, however, were grossly inspected and histopathologically assessed. If treatment-related weight changes had occurred, they would likely have been identified by the detailed histopathological examination.

Methods followed the European and international guidelines:

EC Commission Directive 87/302/EEC of November 18, 1987; Part B: Methods for the determination of Toxicity Sub-chronic Oral Toxicity Test, 90-day repeated oral dose using rodent species; Official Journal of the European Communities No. L 133, pages 8-11, 1988; and OECD Guideline for Testing of Chemicals; Method No. 408: Subchronic Oral Toxicity - Rodent: 90-day study; May 12, 1981.

Result : Results of Reproductive Organ Weight Determinations

1. MALES: There were no statistically significant deviations of the mean absolute or relative testes weights between treated and control animals.

2. FEMALES: the mean absolute weight of the ovaries was statistically significantly decreased (- 17.0%) in the 7200-ppm dose group. The mean relative ovary weight was the lowest in the 7200-ppm dose group (0.035 mg% = - 12.5%); however, this was not statistically significant.

Terminal Body and Reproductive Organ Weights

MALES

Group	Body W	Testes
0	471	3.59
600	460	3.47
2400	458	3.60
7200	452	3.50
15000	428*	3.51

FEMALES

Group	Body W	Ovaries (mg)
0	265	97.5
600	263	89.6
2400	269	93.8
7200	248	80.9**
15000	242*	89.5

Results of Gross Examination of Reproductive Organs:

1. MALES: No gross lesions were noted in the reproductive organs of male rats of any group.

2. FEMALES: One female iin the control group, the low dose group, the low mid and the high mid dose groups revealed slight or moderate dilation of the lumen of one or both horns of the uterus. The dilated areas contained a clear (water-like) fluid. No similar or other gross lesions were recorded in animals of the high dose group.

Results of Histopathologic Examination

In the epididymides of one high dose animal, the only microscopic finding recorded was a minimal, unilateral mononuclear cell infiltration.

In the pituitary gland of one control male and one control female animal, small cysts were noted (location was not specified in the report, however, most likely in the distal/glandular part).

Cystic dilation of the uterus (i.e. one or both horns) was observed in each one female rat of the control, low, low mid and high mid dose groups, whereas two high dose females displayed this finding. The severity was graded as moderate in animals that showed this finding on gross examination (one animal each in the control, low, low mid and high mid dose groups). The two high dose females affected only showed slight dilation, which was not seen on gross examination at necropsy.

No microscopic findings were noted in the adrenal cortex, adrenal medulla, female mammary gland, ovaries, prostate gland, seminal vesicles (including the attached coagulating glands), testes and vagina.

Test substance :

2-Pyrrolidone CAS No. 616-45-5 Purity 99.7%

Conclusion : All organs of the male and female genital tract examined in a "modern" reproduction toxicity study, with the exception of oviducts, were investigated grossly and histopathologically. All other organs required for histopathology in the OPPTS 870.3800 guideline were investigated histopathologically. No gross lesions and no microscopic findings were detected that were indicative of an alteration of male or female reproductive performance. The few gross lesions and microscopic findings reported in these organs were all interpreted as incidental lesions, with respect to both incidence and severity.

Although organ weights for uterus (with oviducts and cervix), epididymides (total weights for both and cauda weight for either one or both), seminal vesicles (with coagulating glands and their fluids), prostate gland, pituitary gland and spleen were not taken, these organs were grossly inspected and

histopathologically assessed. If treatment-related adverse effects had occurred they would have been identified histopathologically or grossly.

In summary, the results of the 90-day subchronic toxicity with 2-Pyrrolidone in male and female Wistar rats are regarded as valid to interpret the potential reproductive performance of the animals as being un-altered by administration of the test article via drinking water.

Reliability : (1) valid without restriction
Guideline study, with good documentation.

Flag : Critical study for SIDS endpoint

03.08.2003 (11)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	days 6-15 of gestation
Frequency of treatm.	:	Daily
Duration of test	:	
Doses	:	190, 600, 1900
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 190 mg/kg bw
NOAEL teratogen.	:	= 600 mg/kg bw
Result	:	Not Specific Developmental Toxin
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	
GLP	:	yes
Test substance	:	

Method :

Groups of 25 pregnant rats were exposed to the test substance by oral gavage using distilled water as vehicle at dose levels of 0, 190, 600 or 1900 mg/kg-bw. On day 20 of gestation, each female was killed and given a gross pathological examination. The gravid uterus was weighed, its contents were examined and all the fetuses were weighed and examined externally. Of these fetuses, approximately half were given a fresh internal examination, their heads removed and examined by the technique of Wilson. The remaining fetuses were eviscerated. All fetuses were stained with Alizarin Red S and their skeletons examined.

Female Sprague-Dawley rats [Crl:CD (SD) BR] were obtained from Charles River Breeding Laboratories, Kingston, New York. After arrival, animals were examined by a veterinary aide; any animals found in poor condition were rejected from the study. After an acclimation period of 14 days, each female was placed in a cage with a proven male breeder of the same strain and source. On the day of mating (Day 0 of gestation), the females were 80-93 days of age and weighed between 231 and 320 g. Pregnancy was assumed when there was positive identification of spermatozoa in the daily vaginal lavage and this was termed day 0 of gestation. Animals were individually housed except during mating.

MATERNAL IN-LIFE DATA: Animals were checked twice daily for mortality and clinical signs. Pregnant females were examined prior to and following dosing for reactions to treatment, indications of poor health and abnormal

behavior from day 6 to day 15 of gestation. Animals were weighed once each week during the acclimatization period and on days 0, 6, 9, 12, 15, 18 and 20 of gestation. Food intake was assessed for all animals on days 0 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18 and 18 to 20 of gestation. On day 20 of gestation, female rats were killed by carbon dioxide asphyxiation followed by exsanguination from the abdominal aorta, each was given a complete gross pathological examination.

MATERNAL EXAMINATION: The reproductive tract of each female was dissected out, the ovaries removed and the corpora lutea counted. The uterus was weighed. The uterine contents were examined and the number and position of live fetuses, dead fetuses, early (endometrial gland with or without some placental tissue), middle (discernible placental and fetal tissue present) and late (fetal structure apparent) resorptions were recorded. The fetuses were then removed from the uterus for examination. The uterus of any animal judged to be nonpregnant was stained with 10% aqueous (v/v) ammonium sulphide solution and was then examined for implantation sites.

FETAL EXAMINATION: Each fetus was weighed, given a detailed external examination with external sex being recorded and then killed. A detailed internal examination using a dissecting microscope was performed on approximately one half of the fetuses, selected randomly from each litter, which were then eviscerated. The heads of these fetuses were removed and placed in Bouin's fluid for examination by the technique of Wilson. The remaining fetuses in each litter were eviscerated; these and the bodies of those fetuses examined internally were placed in 85% ethanol/15% methanol for subsequent staining with Alizarin Red S using a modified Dawson technique for skeletal examination.
Abnormalities were classified as major malformations, minor visceral or skeletal anomalies or common skeletal variants.

STATISTICAL METHODS: The group mean body weights and body weight gains of animals with live fetuses were calculated. The group mean corrected body weights for day 20 of gestation (body weight on day 20 minus gravid uterus weight) and the corrected body weight gains from day 6 to 20 (corrected body weight day 20 minus body weight day 6) were calculated (Data for non-pregnant animals were not included). These parameters were analyzed using one-way analysis of variance, and where the F value was found to be of significance ($P < 0.05$), intergroup differences between control and treated groups were examined using Dunnett's "t" test.

The group mean live litter size, corpora lutea count, number of implants and number of resorptions were calculated. The individual and group litter mean for the sex ratio and pre- and post-implantation losses were calculated. Statistical analyses were performed using the Kruskal-Wallis test and where the "H" value was significant ($P <0.05$) the Mann-Whitney "U" test was used to analyze for differences between control and test groups.

The litter mean fetal weights and group mean fetal weights were calculated and statistical analysis was performed using an analysis of variance (one-way classification) and Dunnett's "t" test.

The incidences of major malformations and minor anomalies were reported as the number of litters with abnormalities in each group and the number of

fetuses affected. Statistical analyses comparing the number of litters (containing major malformations) in each test group with the control values were performed using either the chi-square test (with Yate's correction factor) or Fisher's exact probability test. The incidence of minor anomalies was analyzed in the same manner. The incidence of common skeletal variants was reported as the litter mean percentage of fetuses affected. Statistical analyses were performed by comparing the litter mean percentage incidences of each test group with the control group using the Kruskal-Wallis and Mann-Whitney "U" tests.

Result :

No animals died during the study and no treatment-related clinical signs were reported.
BODY WEIGHT: Between day 6 and day 9 of gestation, the 1,900 mg/kg-day group lost weight while the body weight gains were significantly reduced in the 600-mg/kg-day group. There were significantly reduced body weight gains over the day 9 to 12 interval in the 1,900-mg/kg-day group. These reduced body weight gains resulted in significantly reduced body weights from day 9 to 20 of gestation in both the 600 and 1,900 mg/kg-day groups. The corrected body weights were significantly decreased in the 600 and 1,900 mg/kg-day groups and the corrected body weight gain was decreased significantly in the 1,900-mg/kg-day group.
FOOD CONSUMPTION: (Table 5, Appendix 3)
Over days 6 to 9 and 9 to 12 of gestation, food consumption in both the 600 and 1,900-mg/kg-day groups was significantly reduced. Food consumption continued to be significantly reduced over days 12 to 15 of gestation in the 1,900-mg/kg-day group only.
GROSS PATHOLOGICAL FINDINGS: (Table 1, Appendix 6)
Gross pathological examinations revealed no abnormalities related to treatment other than a few incidental findings among mid and low-dose animals on the study.

UTERINE FINDINGS: (Tables 1 and 8, Appendix 7)
The pregnancy rate was at least 88.0% in all groups. Ammonium sulphide staining revealed no other pregnancies.
Gravid uterus weights were significantly reduced in the high-dose group. There were no significant differences between control and treated groups for the following ovarian and uterine parameters: total corpora lutea, total implantation sites, numbers of male and female fetuses, sex, ratio, number of live fetuses, number of dead fetuses, early, middle and late resorptions, total resorptions and pre- and post-implantation losses.

FETAL FINDINGS:
FETAL WEIGHTS were significantly reduced for males, females and totals only in the high-dose group.
MAJOR MALFORMATIONS,In the high-dose group there was a significant increase in the incidence of litters and fetuses with major malformations with 5 fetuses affected. All had acaudia or microcaudia and anal atresia. In addition, one of these fetuses had absence of some thoracic and all lumbar, sacral and caudal vertebrae and absence of 9 pairs of ribs. The incidence of major malformations in the mid and low-dose groups was not different from controls.
MINOR VISCERAL ANOMALIES: There was no effect upon the overall incidence of litters with minor visceral anomalies, but the incidence of fetuses affected was significantly increased in the high-dose group.
MINOR SKELETAL ANOMALIES: The overall incidence of fetuses with minor skeletal anomalies was significantly increased at the high dose. This increase was primarily the result of significantly increased incidences of

several findings which included reduced ossification of frontal bones, irregular ossification of supraoccipital bones, reduced number of pre-sacral vertebrae and ossification centers on the seventh cervical vertebra. In the mid and low-dose groups, statistically significant differences in the incidences of reduced ossification of the interparietal bone, ossification centers on the first lumbar vertebra, reduced ossification of the pubic bones, reduced ossification of the ischial bones or absent ribs were attributed to intergroup variation.

COMMON SKELETAL VARIANTS:The percentage of fetuses with thoracic centrum variants was significantly decreased in the 1900 mg/kg-day group. There was a statistically significant reduction in the percentage of fetuses with sternebral (5 or xiphisternum) variants in the 190-mg/kg-day group that was attributed to intergroup variation.

The accompanying table presents most of the fetal results in tabular form.

Test substance : 2-Pyrrolidone CAS No. 616-45-5, Purity 99.6%

Attached document : Tab-Dev-01.bmp

Dose(mg/kg)	0	190	600	1900
Dams Pregnant	22	25	23	24
Corpora lutea:	17.5	18.3	17.4	17.5
Implantations:	16.3	16.4	16.4	15.5
Postimplantation Loss:	0.7	0.8	1.0	0.8
Live Fetuses/Litter	15.5	15.5	15.4	14.8
Total # Dead Fetuses	0	0	0	0
Total # Live Fetuses:	341	388	355	354
Mean Fetal Weight (g):	3.45	3.54	3.40	3.12
Sex Ratio (male):	0.43	0.46	0.46	0.51
Major Malformations	0	1	1	5*
Litters with Maj Malf	0	1	1	5*
Minor Visceral Malf.	1	2	1	7
Litters with MVM	1	2	1	5
Minor Skeletal Anoml	82	98	60	140**
Litters with MSA	19	23	19	23

* Statistically Significant

Conclusion :

Treatment of pregnant rats with 2-pyrrolidone, by gavage, at dosages of up to 1,900 mg/kg-day, throughout major organogenesis, resulted in significant maternal toxicity at the 600 and 1,900 mg/kg-day levels, as evidenced by decreased body weights and food consumption. At the 1,900 mg/kg-day level there were increased incidences of major malformations, minor visceral and skeletal anomalies and decreased fetal weights. No effect upon postimplantation loss was observed.
Therefore, 2-pyrrolidone at a dose of 1,900 mg/kg-day was considered embryo- and fetotoxic but not embryolethal. No effect upon embryonic development was seen at the 600 mg/kg/day level where a significant level of maternal toxicity occurred. The 190 mg/kg/day group was considered the no effect level for maternal toxicity. Based upon these data, the A/D (adult/developmental) ratio was calculated to be <1, indicating 2-pyrrolidone did not show selective toxicity to the rat fetus.

Reliability : (1) valid without restriction
Modern Guideline study under GLP

Flag : Critical study for SIDS endpoint

31.12.2002 (15)

Species	:	rat
Sex	:	
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	days 6-15 of gestation
Frequency of treatm.	:	daily
Duration of test	:	10 days
Doses	:	1700 microliters/kg-bw
Control group	:	yes, concurrent no treatment
Result	:	Not teratogenic in the rat by oral gavage
Method	:	other: FDA 1966
Year	:	1971
GLP	:	no
Test substance	:	

Method :

Test substance was administered in distilled water to 25 presumed-pregnant dams on days 6-15 of gestation. Dosing solution was prepared fresh daily. Controls (26 dams) were untreated. Animals were checked daily for adverse clinical signs and mortality. Animals were weighed three times a week during the dosing period. The dose of the test substance was based on the weight of the rat on day 0. The concentration of the solutions was adjusted in such a way that the amount of test substance to be administered for 100 g body weight was contained in a volume of 0.5 ml. On the 20th day of post coitum all the animals were sacrificed, the uteri were removed, the implantation and resorption sites were recorded, the number of live and deed fetuses, their body length, their weight and sex, and the weight of the placentas were determined. The fetuses were examined macroscopically for any malformations. A third of the fetuses of each dam were fixed in Bouin's solution and transversal sections were prepared and assessed according to Wilson's method (Wilson, Warkany: Teratology, Principles und Techniques, 1965). For the assessment of the skeletal system, the remaining fetuses were fixed in 96% strength alcohol, clarified with potassium hydroxide solution and stained with Alizarin red-S using a modified Dawson method. The uteri of the apparently nonpregnant animals or the empty uterine horns in the case of single-horn pregnancy were stained in 10% strength ammonium sulfide solution and then assessed again in order to determine early resorptions.

Remark :

The dose level was 1700 microliters/kg-bw. Based on the specific gravity of 1.103, this is approximately 1875 mg/kg-bw.

Without the maternal body-weight gain data the maternal toxicity cannot be adequately assessed. This dose was approximately the same a as that used in the three-dose level 1990 developmental toxicity study and the results are similar in that there was not a major teratogenic effect.

Result :

All the pregnant rats tolerated the 10 oral administrations of test material without visible signs of toxicity. One dam died on the 17th day post coitum. The animal proved to be not pregnant. No substance-induced changes could be observed macroscopically. The mean number of implantations and the percentage of resorptions did not differ between the test and control groups. Maternal weights, although recorded, were not included the report.

MACROSCOPIC FETAL EFFECTS: The mean weight and length of the fetuses in the test group did not differ from the values in the control group.

The mean weights of the placentas in the test group and untreated control group were also comparable. The percentage of malformed live fetuses was 2.8 in both groups; similarly, the percentage of runts was the same in the test and control groups.

SKELETAL ASSESSMANT: In treated animals, one fetus (dam No. 6) had a bipartite 12th thoracic vertebral centrum. One fetus (dam No. 10) was observed to have anasarca and two other fetuses of this dam had a cleavage of the eleventh thoracic vertebral centrum. Dam No. 22 had one malformed fetus. The tail of this fetus was missing and atresia was also reported. One fetus of dam No. 24 had a bipartite eleventh thoracic vertebral centrum.
In Untreated animals: One fetus (dam No. 30) had a bipartite eleventh thoracic vertebral centrum. One fetus (dam No. 33) had a bipartite twelfth thoracic vertebral centrum. One fetus of each of dams Nom. 34 and 35 had a bipartite eleventh thoracic vertebral centrum. The presphenoid was missing in one fetus of dam No. 44. One fetus of dam No. 47 had a bipartite 12th thoracic vertebral centrum.

TRANSVERSE SECTIONS: No malformations were found in the fetuses of test or control animals.

Test substance : 2-Pyrrolidone CAS No. 616-45-5

Conclusion : The pregnant dams tolerated the 10 oral administrations of test material without any visible symptoms of toxicity or any macroscopically evident pathological changes. The malformations or anomalies found in the fetuses of the test group corresponded in type and number to those of the controls and historical controls. The test material does not have teratogenic effects in Sprague-Dawley rats.

Reliability : (2) valid with restrictions
A reliability of 2 is assigned. Although some important details are lacking this study was conducted according to a standard procedure that is scientifically defensible. It has value as a supporting study.

08.12.2002 (3)

(1) All flatworms survived the 96-hour exposure period.

(2) BASF AG, Abt. Toxikologie, unpublished study report (86/286), 26.11.1987

(3) BASF AG, Abt. Toxikologie, unveroeffentlichte Untersuchung (XIX/421), 04.08.1971

(4) BASF AG, Abteilung Toxikologie; unpublished report. Cytogenetic Study In Vivo of Pyrrolidon-2 in Mice, Micronucleus test. (92/1491), 28.06.93

(5) BASF AG, Abteilung Toxikologie; unveroeffentliche Untersuchungen (79/409), 09.04.1981

(6) BASF AG, Analytisches Labor; Unpublished Stiudy (J.Nr.129300/04 vom 14.06.88)

(7) BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung (Pyrrolidon dest., 1977)

(8) BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0701/88)

(9) BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0701/88, Fa.Noack)

(10) BASF AG, Report of the Subchronic oral toxicity with 2-Pyrrolidone in Wistar rats, 3-month drinking water, Project No. 52S0014/92038 June 4, 1998

(11) BASF AG, Report of the Subchronic oral toxicity with 2-Pyrrolidone in Wistar rats, 3-month drinking water, Project No. 52S0014/92038 June 4, 1998.

Dr. med. vet. C. Gembardt, Special Report: "Assessment of the reproductive performance of 2-Pyrrolidone in male and female Wistar rats from data obtained in a subchronic toxicity study" 15 July 2003

(12) BASF AG: Abt. Toxikologie, unpublished report, (92/14), 01.08.1995

(13) BASF AG: Abt. Toxikologie, unveroeffentlichte Untersuchung,(XI/407), 07.11.1961

(14) BASF Labor Okologie, unpublished study, 28.06.88

(15) Bio-Research Laboratories Inc, An Oral Teratoloty Study of 2-Pyrrolidone in the Rat. Project # 83880, Dec. 19, 1990 Sponsored by GAF Chemicals and BASF AG

(16) Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996. 1378

(17) Chem Inspect Test Inst; Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan; Published by Japan Chemical Industry Ecology-Toxicology & Information Center. ISBN 4-89074-101-1 p. 5-5 (1992)

(18) Daubert, T.E. and Danner, R.P. Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. Design Institute For Physical Property Data, American Institute Of Chemical Engineers. Hemisphere Pub. Corp., New York, NY., 5 Vol, 1997

(19) EPIWIN 3.05 caluclation SRC Syracuse NY

(20) Estimated using HYDROWIN 1.67 as found in EPIWIN 3.05, SRC Syracuse NY

(21) Flick, E.W. (ed.). Industrial Solvents Handbook 4 th ed. Noyes Data Corporation., Park Ridge, NJ., 1991. 918, as cited in Hazardous Substance Data Base, NLM, Revison of 8-6-2002

(22) Jagannath, D.R., Mutagenicity Test on 2-Pyrrolidone in the Ames Salmonella/Microsome Reverse Mutation Assay, Final Report, Hazleton Labs, GAF Sponsor April 24, 1987.

(23) Mayer, V.W. Goin, C. J. and Taylor-Mayer, R. E. Aneuploidy Induction in Saccharomyces cerevisiae by Two Solvent Compounds, 1-Methyl-2-pyrrolidinone and 2-Pyrrolidinone. Environmental and Molecular Mutagenesis 11:31-40, 1988

(24) MB Research Laboratories Inc project number MB-92-1432 Sponsored by International Specialty Products, 4/29/1992.

(25) Perry, C.M., Smith,S.B. Toxicity of Six Heterocyclic Nitrogen Compounds to Daphnia pulex. Bull. Environ. Contam. Toxicol.41, 604-608, (1988)

(26) Riddick, J.A.; Bunger, W.B.; and Sakano, T.K. Organic Solvents: Physical Properties And Methods Of Purification. Techniques Of Chemistry. 4th Ed. New York, NY: Wiley-Interscience. 2: Pp.1325, 1986 (as cited in CIS 4-2002)

(27) Submission to U.S. EPA: Raw data for ecotoxicity information on 2-Pyrrolidinone (CAS Reg No 616-45-5), with cover letter dated 01/29/86 Source: EPA/OTS; Doc #FYI-OTS-0794-1152 Submitted by Eastman Kodak Company