FOREWORD INTRODUCTION

Imidazole

CAS N°: 288-32-4

SIDS Initial Assessment Report

For

SIAM 17

Arona, Italy, 11 – 14 November 2003,

1.	**Chemical Name:**	Imidazole
2.	**CAS Number:**	288-32-4
3.	**Sponsor Country:**	Germany Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Contact person: Prof. Dr. Ulrich Schlottmann Postfach 12 06 29 D- 53048 Bonn- Bad Godesberg
4.	**Shared Partnership with:**	BASF AG, Germany; Air Products and Chemicals, Inc., USA
5.	**Roles/Responsibilities of the Partners:**	
∉	Name of industry sponsor /consortium	BASF AG, Germany Contact person: Dr. Hubert Lendle, GUP/CL - Z570 D-67056 Ludwigshafen
∉	Process used	see next page
6.	**Sponsorship History**	
∉	How was the chemical or category brought into the OECD HPV Chemicals Programme ?	by ICCA-Initiative
7.	**Review Process Prior to the SIAM:**	last literature search (update): 09 July 2003 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms 09 April 2003 (Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms
8.	**Quality check process:**	As basis for the SIDS-Dossier the IUCLID was used. All data have been checked and validated by BUA.
9.	**Date of Submission:**	August 14, 2003
10.	**Date of last Update:**	

11. Comments: **OECD/ICCA – The BUA* Peer Review Process**

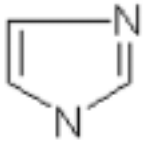
Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA guidelines/instructions for the OECD/ICCA peer review process and includes:

- a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
- Review of data and assessment of the quality of data
- Review of data evaluation
- Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications
- Review of key study description according robust summaries requirements; completeness and correctness is checked against original reports/publications

  (if original reports are missing: reliability (4), i.e. reliability not assignable)
- Review o f validity of structure-activity relationships
- Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)
- In case of data gaps, review of testing plan or rationale for not testing

* BUA (GDCh-Beratergremium für Altstoffe): Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	288-32-4
Chemical Name	Imidazole
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Imidazole is readily absorbed and excreted in humans and in test animals after oral and rectal administration. Peak plasma levels are reached within 15 to 30 minutes in rats and within approx. 3 hours in humans. Elimination half-life in humans is approx. 2 to 3 hours. Therefore a potential for bioaccumulation is unlikely. Induction of microsomal P450 enzyme in the liver cells of rats and rabbits is restricted to certain isoenzymes such as 7-ethoxycoumarin-O-deethylase and isoenzyme 3a. However, no such induction was seen in the Syrian golden hamster.

Imidazole is of moderate oral toxicity in a scientifically valid study. LD_{50} in rats was determined to be 960-970 mg/kg body weight. 80% Imidazole is corrosive to skin under occlusive conditions. Imidazole is irritating to rabbit eye when tested according to OECD TG 405. Persistent large size cornea opacity indicates the potential of severe eye injury after eye contact. No sensitization study is available.

Liver and kidney are target organs in subacute and subchronic (OECD TG 408) rat studies at dose levels of 180 mg/kg body weight per day and above. Slight centrilobular liver cell hypertrophy and relative liver weight increase was noted. Diffuse ζ2u-microglobulin accumulation was noted in the proximal tubules of the renal cortex only in male rats but was considered to a species-specific effect. The NOAEL was approximately 60 mg/kg body weight per day. Red blood cells were additionally affected in 28-d experiments. Female rats receiving 125 mg/kg body weight per day or more and male rats receiving 500 mg/kg body weight per day were affected. The NOAEL was approximately 62.5 mg/kg body weight per day. This finding was, however, not confirmed in the 90-day guideline study when rats received up to 180 mg/kg body weight per day.

Imidazole was not mutagenic in bacterial test systems generally meeting OECD TG 471 with the Salmonella typhimurium strains TA 98, TA 100, TA 1535, or TA 1537, with or without the presence of metabolic activation by S-9 mix containing rat liver microsomes, with or without preincubation. Imidazol did not induce Unscheduled DNA Synthesis in rat primary hepatocytes in a study equivalent to the OCED TG 482. It was not clastogenic in the mouse micronucleus test according to the OECD TG 474 when imidazole hydrochloride was tested in vivo. The salt dissociates into protonated imidazole and chloride in the stomach following oral gavage.

No reproductive toxicity studies are available. However, no changes of the male and female reproductive organs including sperm quality were noted in a rat subchronic 3-months study according to OECD TG 408 imidazole was given by gavage at 20, 60, and 180 mg/kg bodyweight per day.. The NOAEL for these endpoints was 180 mg/kg body weight per day. In a study conducted in accordance to OECD TG 414 imidazole was developmental toxic and teratogenic at a dose of 180 mg/kg body weight per day showing some maternal toxic effects which is not likely to be the sole cause of the teratogenic effect. The incidence of external and skeletal malformations were significantly increased up to 10%. Furthermore there were soft tissue variations observed. The NOAEL was 60 mg/kg body weight per day for maternal toxicity, developmental toxicity, and teratogenicity.

No studies concerning the long-term toxicity and/or carcinogenic potential of Imidazole are available. It is, however, mentioned that imidazole was negative in the mouse fibroblast cell transformation test.

Environment

Imidazole is a colourless – yellow solid with an amine-like odour. It has a water solubility of 663 g/l at 20 ∀C (pH 10.5 for 68 g/l at 20 °C). Imidazole is a heterocyclic compound containing two nitrogens with pK_a = 7.0 and pK_a =14.9. The melting point ranges from 88.3 to 89.9 ∀C, the boiling point is at 267.8 ∀C at 1013.3 hPa and the vapour pressure is 0.00327 hPa at 25∀C.
The distribution of the substance between the compartments of air, biota, sediment, soil and water was calculated according to Mackay Level I fugacity model. The non-charged molecule distributes mainly to the water (99.98 %).
A soil adsorption coefficient (K_{oc}) of 9.72 was estimated for imidazole. This K_{oc} value suggests that this compound would be mobile in soil and adsorption to suspended solids would not be important. From the pK_a-value of 14.9 it can be assumed that under environmental conditions the substance is available as a cation. Therefore, binding of the substance to the matrix of soils with high capacities for cation exchange (e.g. clay) cannot be excluded. However, no data was available for ionic-ionic interactions in soil. The calculation of a Henry's law constant with the model HENRYWIN 3.1 yields a value of 0.38 Pa*m³/mol at 25 °C. Using the above described values for water solubility and vapor pressure, a Henry's law constant of 0.000034 Pa*m³/mol can be calculated. This low value and the water solubility of imidazole suggest that volatilization from water would not be an important fate process. Hydrolytic degradation is not to be expected under environmental conditions.
The half-life for photodegradation in air was calculated to be 10.7 hours. Half-lives for photolysis in water between 4.4 hours and 307 days have been reported dependant on OH concentrations and light intensity. The substance has no considerable potential for bioaccumulation (log K_{ow} = -0.02, measured). The compound is readily biodegradable (OECD 301 A, 98% after 18 days 10d-window fulfilled). The EC_{50} (30 min) for activated sludge was determined to be >1000 mg/L.
The following aquatic effect concentrations are available:
Leuciscus idus LC_{50} (48 h) = 284 mg/l (nominal concentration).
Daphnia magna: EC_{50} (48 h) = 341 mg/l (nominal concentration).
Scenedesmus subspicatus: E_rC_{50} (72h) = 133 mg/l, with a NOEC of 25 mg/l (corresponding values for biomass are 127 and 10 mg/l respectively; nominal concentration)
Pseudomonas putida EC_{50} (17 h) = 1175 mg/L (nominal concentration)
Tetrahymena pyriformis : IGC_{50} (48 h) = 680 mg/L (nominal concentration)
Although no analytical monitoring of the test substance concentration was performed, and the substance is subject to photolysis in water, it is assumed that the effect values will not be below 100 mg/l.
Using the aquatic toxic effect on the most sensitive species, *Scenedesmus subspicatus*, of 133 mg/l for endpoint growth rate (127 mg/l endpoint biomass) a PNECaqua of 133 µg/l is derived by applying an assessment factor of 1000 according to the EU Technical Guidance Document. This factor is justified, because only short-term toxicity values were available.

Exposure

In 2002, the estimates for imidazole for the world market amounted to approx. 1000– 5000 tonnes/year. The substance was not imported into the European Union in 2002.
The organic compound is used in the chemical industry as an intermediate in the production of pharmaceuticals, pesticides, dye intermediates, auxiliaries for textile dyeing and finishing, photographic chemicals and corrosion inhibitors.
The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine and nucleic acid. Therefore, the bulk of imidazole produced is used in the preparation of biologically active compounds.
According to Swiss, Danish, Finnish, Swedish and French Product Registers imidazole is contained in a large number of products. Due to Swiss information consumer products contain imidazole in concentrations up to 10 %. Danish, Finnish, and Swedish Product Register did not confirm that information regarding consumer products.
There is a potential of releases into the environment during the production and processing of imidazole as an intermediate, and – according to the cited applications in the European Product Registers – from the formulation, processing and use of products containing the substance. During production and internal processing, less than 5 kg/a were emitted into the air by the German producer. Monitoring data of the substance in the sewage and effluent of waste water treatment plants are not available. The exposure of workers at the German production and internal processing plants is controlled. Imidazol is produced under closed system conditions. Exposure measurements at workplace were in a range between 0.21 mg/m³ and 0.32 mg/m³. From the reported use in consumer products, it can be concluded that most of the imidazole is released into wastewater, but part of it may also be released into the atmosphere.

RECOMMENDATION

Human Health: The chemical is a candidate for further work.
Environment: The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health:
The chemical possesses properties (corrosivity to skin, irreversible damage to eyes, teratogenic effects) indicating a hazard for human health. Humans are exposed by consumer products (chemical concentrations up to 10%) and at the workplace. Therefore, the chemical is a candidate for further work. An exposure assessment and if indicated a risk assessment is recommended.

Environment:
The chemical is currently of low priority for further work because of its low hazard potential.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 288-32-4
IUPAC Name: Imidazole
Molecular Formula: $C_3H_4N_2$
Structural Formula:

Synonyms: 1,3-Diaza-2,4-cyclopentadiene

1,3-Diazole

1H-Imidazole (9CI)

Glyoxalin

Glyoxaline

Imidazol

Imidazole (8CI)

Imutex

Methanimidamide, N,N'-1,2-ethenediyl-

Miazole

1.2 Purity/Impurities/Additives

Substance type: organic

Physical status: solid

Degree of purity: ∅99.5 % w/w

Known impurities: Ω0.3 % 2-methylimidazol

Ω0.3 % 4-methylimidazole

Ω0.3 % 1-(methoxymethyl)-1H-imidazole

Ω0.3 % high-boiling components

Ω0.2 % water

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference/comment
Physical state	colourless to pale yellow crystalline flakes with a weak amine-like odor	BASF AG, 1997
Melting point	88.3 - 89.9 °C	BASF AG, 1989a; BASF AG, 1987a; BASF AG, 1991
Boiling point	267.8 °C, 268.1 °C at 1013.3 hPa	BASF AG, 1987b; BASF AG, 1987d
Relative density	1.111 g/cm^3 at 95 °C	BASF AG, 1989b
Vapour pressure	0.00327 hPa at 25 °C	BASF AG, 1987c
Water solubility	663 g/l at 20 °C (pH 10.5 for 68 g/l at 20 °C)	BASF AG, 1988a
Partition coefficient n-octanol/water (log value)	–0.02 at 25 °C	BASF AG, 1988b
Henry's law constant	0.38 Pa*m³/mol at 25 °C (calculated via HENRYWIN v3.10) 0.000034 Pa*m³/mol	BASF AG, 2002a calculated using the above described values for water solubility and vapor pressure
Organic carbon/water partition coefficient, Koc	9.72	BASF AG, 2002a
Viscosity	2.696 mPa*s at 100 °C	Ullmann, 2000
Dissociation Constant	pKa = 14.9 pKa = 7.0	Ullmann, 2000

Imidazole is a heterocyclic compound containing two nitrogens. It is a moderately strong base (pK_a = 7.0), and a weak acid (pK_a = 14.9) (Ullmann, 2000). Imidazole is not ionised significantly between the two pK_a values. The pK_a of 7 represents gaining a hydrogen ion (basicity) and the pK_a of ca. 14 represents loss of hydrogen (acidity).

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

Of the many known methods for producing imidazoles, only the following are of industrial importance (Ullmann, 2000):

(1) the Radziszewski Reaction: a 1,2-dicarbonyl compound is condensed with an aldehyde and ammonia (R1 = H) in a molar ratio of 1:1:2, respectively. The reaction is usually carried out in water or a water - alcohol mixture at 50 - 100 °C. Work-up may involve the usual processes (e.g., distillation, extraction, and crystallization).

(2) Dehydrogenation of D2-Imidazolines: D2-Imidazolines can be obtained by several routes from 1,2-diamino compounds and carboxylic acid derivatives: 1) by reaction of a diamine with a carboxylic acid over an acidic heterogeneous catalyst (e.g., alumina - phosphoric acid) in the gas phase; 2) by reaction of a diamine with a carboxylic acid nitrile in the presence of sulfur or copper

salts in the liquid phase; or 3) by preparation of diformyl derivatives from a diamine and formic acid esters, followed by conversion to imidazoline in the gas phase at 200 - 350 °C over a heterogeneous catalyst (e.g., zinc oxide - alumina).

The D2-imidazoline is dehydrogenated in the gas phase over a precious metal at ca. 300 °C or on alumina - zinc oxide at 400 - 500 °C.

In the year 2002, the global production volume of imidazole is estimated to approx. 1000 - 5000 tons. No other information is available about the production volume in Europe, America or Asia.

The substance was not imported into the European Union in 2002.

Imidazole is produced under closed system conditions (BASF AG, 2003c). Imidazole is used in the chemical industry as an intermediate in the production of pharmaceuticals, pesticides, dye intermediates, auxiliaries for textile dyeing and finishing, photographic chemicals and corrosion inhibitors.

The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine and nucleic acid. Therefore, the bulk of imidazole produced is used in the preparation of biologically active compounds (Ullmann, 2000).

Additional applications are cited in the European Product Registers.

According to the Swiss, Danish and Swedish Products Registers, imidazole is contained in a large number of products, some of them may be available to consumers.

In 2001 in the Swedish Product Register there were 13 products on the Swedish market with a total amount of imidazole of 5 tons/a. (Swedish Product Register, 2003).

The Danish Product Register cited 18 products that contain imidazole in a total amount of 1 tons/a. 12 products have a content of imidazole of up to 2 %, 4 products a content of 2 – 20 %. Product types were process regulators and anti-freezing agents (Danish Product Register, 2003).

In the Swiss Product Register there were registered 51 products with 48 professional and 3 with consumer applications. Approx. 40 % of the products were used in photographic and laboratory applications by professional industry. Further applications were glues/adhesives, cement filler or sealing compounds (approx. 10 %), paints, varnishes and lacquers (approx. 8 %), anti-freezing agents (approx. 8 %) and auxiliary and supplementary agents (approx. 8 %). Three (approx. 6 %) of the registered products were available to consumers as cleaning agents, washing agents and soap and special applications for swimming-baths (Swiss Product Register, 2002).

In the Finnish Product Register 4 products containing imidazole in a content of 1 – 10 % are registered. The total quantity of imidazole in these products in the year 2001 was 1 ton. The most frequent use category reported was: photo chemical. The most frequent industrial uses reported are: publishing, printing and reproduction of recorded media (Finnish Product Register, 2003).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

There is a potential of releases into the environment during the production and processing of imidazole as an intermediate, and - according to the cited applications in the European Product Registers - from the formulation, processing and use of products containing the substance. A non-quantifiable exposure of the terrestrial compartment may occur from possible residual contents of

imidazole in the subsequent products that are used as pesticides. However, no information is available on this point.

No analytical monitoring of imidazole in the effluent of the waste water treatment plant of BASF AG takes place (BASF AG, 2002b).

During production and internal processing at BASF AG, Ludwigshafen (Germany), less than 5 kg imidazole were emitted into the air in 2000 (BASF AG, 2002c).

Emission data from other production and processing sites was not available.

For the uncharged molecule, modelling using Mackay, Level I indicates that water is the main target for environmental distribution (99.98 %; BASF AG, 2002d). It has to be considered that under environmental conditions around 50 % of the substance is expected to be in cationic form. No data are available to estimate the environmental distribution of the charged molecule.

A soil adsorption coefficient (K_{oc}) of 9.72 (log K_{oc} = 0.99) was estimated for imidazole via PCKOCWIN v1.66 (BASF AG, 2002a). The K_{oc} value suggests that adsorption to suspended solids is not to be expected. From the pK_a-value of 14.9 it can be assumed that under environmental conditions the substance is available as cation. Therefore, binding of the substance to the matrix of soils with high capacities for cation exchange (e.g. clay) cannot be excluded. However, no data was available for ionic-ionic interactions in soil. The calculation of Henry´s law constant via HENRYWIN v3.10 yields a value of 0.38 Pa* m^3/mol at 25 °C (BASF AG, 2002a). Using the above described values for water solubility and vapor pressure, a Henry's law constant of 0.000034 Pa*m^3/mol can be calculated. This low value and the water solubility (663 g/l at 20 °C; BASF AG, 1988a) of imidazole suggests that volatilization from water would not be an important factor in the environmental distribution process. In addition, due to the protonation of the substance under environmental conditions, the volatility is further reduced. The half-life for photodegradation in air was calculated via AOP v1.90 to 10.7 hours (BASF AG, 2002a). Due to the measured partition coefficient (log K_{ow} -0.02; BASF AG, 1988b) an accumulation in organisms is not to be expected.

Imidazole is readily biodegradable according to the results obtained in a test conducted according to OECD 301 A (98 % degradation after 18 days, 10d-window fulfilled; BASF AG, 2003a).

Based on the chemical structure of the substance hydrolysis is not to be expected under environmental conditions. The half-life for photooxidation in water was measured to 32 hours (indirect photolysis, reaction with singlet oxygen as sensitizer; Haag and Hoigné, 1986). Other references report half-lives for indirect photolysis in water by reaction with OH as sensitiser in the range of 4.4 hours (summer, midday, shallow water body) to 307 d (for entire water column of 14 m) (dependent on the OH concentration and light intensity) (Rao et al., 1975; Zepp et al., 1987; Mill, 1999). However, as imidazole is readily biodegradable, this degradation process is not relevant for surface waters.

2.3 Human Exposure

2.3.1 Occupational Exposure

Exposure measurements (n = 3) at workplace have been performed at the production site at BASF AG, Ludwigshafen (Germany) between 1996-2002: the measured concentrations were in a range between 0.21 mg/m^3 and 0.32 mg/m^3 (BASF AG, 2002e).

2.3.2 Consumer Exposure

Due to the information from European product registers, exposures to consumers and workers are likely. In the Swiss Product register, 51 products, among them 3 consumer products, containing imidazole are listed. The highest concentration (up to 10 %) is reported for one cleaning agent (Swiss Product Register, 2002). According to the current Swedish Register, there were 13 products, among them 1 consumer product, on the Swedish market containing imidazole (Swedish Products Register, 2003). Fore more information see chapter 2.1.

2.3.3 Other Information on Human Exposure

To date it is not clear if the drug described in one publication as an experimental drug has reached the market (limited access to these data and proprietary rights for pharmaceuticals has to be taken into account). Pharmaceutical uses are handled by FDA or the respective EU directorate.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Imidazole reached peak plasma levels within 15 to 30 minutes in rats dosed with approx. 17 mg/kg bw and disappeared within 4 hours. Similar results were obtained for imidazole with ITF 182, a novel drug called Selezen that consists of the salt of protonated imidazole and 2-hydroxybenzoate in 1:1 molar quantities, with doses containing up to 66 mg imidazole/kg bw (Pagella et al., 1983). The pharmacokinetic parameters were determined in human studies with ITF 182 in single (248 mg of imidazole) and multiple dose (3 single doses per day) studies. The pharmacokinetic parameters were comparable between different experiments, i.e. single or multiple dosing, oral or rectal applications, or oral tablet or oral drops did not exert marked influences. The main pharmacokinetic parameters in humans after oral intake may be summarized as follows: maximum plasma levels were reached after approx. 3 hours, elimination half-life was approx. 1.8 to 3 hours. Bioavailability was complete. Protein binding was determined to range between 5 to 15 %. In contrast, no effects were noted in a pilot study after dermal application (Noseda et al., 1988; Kuemmerle et al., 1987).

Response of enzymes involved in liver drug metabolism to imidazole treatment was determined in several studies. No increase in total microsomal P450 content was observed after a 4 day i.p. administration of 200 mg/kg bw per day in female Sprague-Dawley rats. Statistically significantly increased activities of 7-ethoxycoumarin-O-deethylase (1.7-fold) and Aminopyrine-N-demethylase (1.26-fold) were noted whereas aniline and p-nitrophenol hydroxylases were insignificantly reduced (Reinke et al., 1985). New Zealand White rabbits treated with imidazole (200 mg/kg bw, 4 days) showed increased total p450-content in liver (1.24-fold) compared with controls and a 4.47-fold increase of the isozyme 3a (Koop et al., 1985). No significant changes were noted in pretreated Syrian Hamsters of both sexes (200 mg/kg bw, 4 days) with respect to relative liver weight, total microsomal p450-content, microsomal and cytosolic enzyme activities involved in phase I (demethylation of p-nitroanisole and ethylmorphine, NADPH-Cytochrome C-reductase) and phase II drug metabolism (sulfotransferase, glutathione transferase) (Ritter and Franklin, 1987).

Conclusion

Imidazole is readily absorbed and excreted in humans and in test animals after oral and rectal administration. Peak plasma levels are reached within 15 to 30 minutes in rats and within approx. 3

hours in humans. Elimination half-life in humans is approx. 2 to 3 hours. Therefore a potential for bioaccumulation is unlikely.

Induction of microsomal P450 enzyme in the liver cells of rats and rabbits is restricted to certain isoenzymes such as 7-ethoxycoumarin-O-deethylase and isoenzyme 3a. However, no such induction was seen in Syrian golden hamster.

3.1.2 Acute Toxicity

Studies in Animals

Inhalation

There is no inhalation toxicity study nor acute dermal study.

Oral

The acute oral toxicity is moderate: in a non-guideline study a LD_{50} in rats was determined to be 960 - 970 mg/kg bw (BASF AG, 1956). The symptoms were described as convulsions and disequilibria with lateral posture. Deaths occurred within one day. Apathy and accelerated respiration was noted in survivors. There was no difference if pure (100 %) or technical (95%) imidazole was tested as 10 % aqueous preparation having a pH of 9.

In a limited study a LD_{50} in male mouse was determined to be 1180 mg/kg bw (Nishie et al., 1969).

Conclusion

Imidazole is of moderate oral toxicity in a scientifically valid study. LD_{50} in rats was determined to be 960 - 970 mg/kg bw.

3.1.3 Irritation

Skin Irritation

When 80 % Imidazole was applied to the intact rabbit skin as an aqueous paste for 1 or 4 hours under occlusive dressing skin reactions were noted as early as one hour after removal of the dressing. Focal necrosis developed overnight in all animals and was described as leather-like at the end of the observation period when pathology confirmed full thickness necrosis after sacrifice of the animals (BASF AG 1956, 1979a).

Eye Irritation

Application of 0.1g unchanged imidazole to the rabbit's eye (Draize test; according to Federal Register 38 no. 178 § 1500.42 (1973)) affected conjunctiva, cornea, and the nictating membrane of the animals. Grade 2 reddening and swelling of the conjunctiva was noted along with chemosis which aggravated and persisted from grade 1 after 24 hours to grade 3 until day 8. A slight grade 2 cornea opacity persisted until the end of the observation period on day 8. The affected corneal area comprised more than 3/4 (BASF AG, 1979b).

Grade 3 cornea opacity and grade 2 effects on iris was observed in a guideline study (OECD TG 405) referenced by ECETOC using the grading scale according to Draize. The findings persisted on day 14 after application of imidazole (ECETOC, 1998).

Conclusion

80 % Imidazole is corrosive to skin under occlusive conditions. Imidazole is irritating to rabbit eye when tested according to OECD TG 405. Persistent large size cornea opacity indicates the potential of severe eye injury after eye contact.

3.1.4 Sensitisation

No study available

3.1.5 Repeated Dose Toxicity

Subchronic effects of imidazole were determined in a 90-day study in Wistar rats according to OECD TG 408. This study aimed at the examination of systemic and specific organ toxicity, ophthalmologic effects, effects on male and female reproductive organs, and effects on behaviour and sensi-motor capabilities which were examined in a series of tests delineated as the Functional Observation Battery, FOB. Imidazole was given daily by gavage dissolved in water at 20, 60, and 180 mg/kg bodyweight per day. Liver and the male kidney were identified as target organs in the animal groups receiving 180 mg/kg bw per day as substantiated by significantly increased relative liver weights in males (+7.5 %) and females (+2.6 %) which correlated with minimal to slight centrilobular liver cell hypertrophy in males (9/10 animals affected) and females (2/10), and by a significant increase of absolute and relative kidney weight in high-dose males that was accompanied by an accumulation of ζ2u-microglobulin in the epithelia and lumina of the proximal tubules of the male rat renal cortex. The Ŭ2u-globuline was detected by Mallory Heidenhain staining technique specificity for Ŭ2u-microglobulin could be demonstrated by immunohisto-chemical staining. The accumulation of microglobulin is considered as a rat-specific phenomenon and has no toxicological relevance for humans. Additionally, significant changes in parameters of blood chemistry were noted in high dose animals as substantiated by decreased serum globulin and chloride in male rats, and total protein, albumin, globulin, and chloride in females.

No other substance-related effect was noted in the 90-d study at 180 mg/kg bw per day; i.e. mortality, clinical observation for signs of toxicity, bodyweight, bodyweight development and food consumption, clinical chemistry other than noted above, pathology and histopathology of the numerous organs examined were not affected. Also, no effects were noted during ophthalmologic examinations or the FOB tests. Male and female reproductive organs were not affected (including histopathology), as were sperm quality parameters (sperm number, motility, and morphology were determined in testis and epididymides). No substance-related effect was noted at the intermediate and at the low dose level. Therefore the no observed adverse effect level (NOAEL) was 60 mg/kg bw per day in both sexes under the conditions of this study (BASF AG, 2002g).

In addition to the liver and the kidney, red blood cells were identified as a target in a 4 week rat study (oral gavage, groups at 0, 62.5, 125, 250, and 500 mg/kg bw per day) in Sprague Dawley rats when hemoglobin was significantly decreased in females at a dose of 125 mg/kg bw per day ($p<0.05$) and above ($p<0.001$). Hematocrit and the numbers of erythrocytes were also significantly decreased ($p<0.05$) in females at a dose of 250 mg/kg bw per day and above. In male rats hemoglobin and hematocrit were significantly reduced ($p<0.05$) only at the high dose. The effect on red blood cells was confirmed in preliminary investigations (not reported) of the 90 day study when a 4 week exposure of Wistar rats showed similar results at comparable (high) dose levels of 250 mg/kg bw and above. The marginal effects in the old 4 week study at 125 mg/kg bw should not be regarded as substance related, as they were not confirmed even after 90 day exposure in the new study at higher dose levels (180 mg/kg bw per day; BASF AG, 2002g). Relative mean liver weights were significantly ($p<0.01$) increased in male and female rats at 125 mg/kg bodyweight per day and

more. Relative kidney weight was only increased in male rats at 250 mg/kg bw per day at the same level of probability. The NOAEL was 62.5 mg/kg bw per day (BASF AG, 1976).

It should, however, be noted that effects on red blood cells was not confirmed in the rat, oral gavage 90d-guideline study at any dose level including the highest tested (180 mg/kg bw per day; BASF AG, 2002g).

Conclusion

Liver and kidney are target organs in subacute and subchronic (OECD TG 408) rat studies at dose levels of 180 mg/kg bw per day and above. Slight centrilobular liver cell hypertrophy and relative liver weight increase was noted. Diffuse ζ2u-microglobulin accumulation was noted in the proximal tubules of the renal cortex only in male rats but was considered to a species-specific effect. The NOAEL was approximately 60 mg/kg bw per day.

Red blood cells were additionally affected in 28-d experiments. Female rats receiving 125 mg/kg bw per day or more and male rats receiving 500 mg/kg bw per day were affected. The NOAEL was approximately 62.5 mg/kg bw per day. The effect on red blood cells was, however, not confirmed in the 90-day guideline study when rats received up to 180 mg/kg bw per day.

3.1.6 Mutagenicity

Studies in Animals

In vitro Studies

Imidazole was tested in the standard Ames test and in the preincubation Ames test conducted under GLP and according to the OECD TG 471. The substance was tested with *Salmonella typhimurium* TA 1535, TA 100, TA 1537, and TA 98 both in the presence and absence of metabolic activation in concentrations up to 5000 µg/plate. No mutagenic or bacteriotoxic effect was noted (BASF AG, 1992).

Imidazole and its metabolites hydantoin, hydantoic acid, and N-acetyl-imidazole were also negative in a standard-plate Ames-test equivalent to the OECD TG 471 with *S. typhimurium* TA 97, TA 98, TA 100, and TA 102 in the presence and absence of metabolic activation. Test substance concentrations were up to and including 10 000 µg/plate without reaching cytotoxicity (Forster et al., 1992).

Imidazole did not induce Unscheduled DNA Synthesis (UDS) in rat primary hepatocytes in a guideline study conducted equivalent to the OECD TG 482. The test substance concentrations (0.25, 0.5, 1, 2, 4 mg/ml) reached the cytotoxic concentration range. Cell survival was 50 % at 1 mg/ml (Forster et al., 1992).

In vivo Studies

Imidazole hydrochloride was tested in a micronucleus test in accordance with the OECD TG 474 under GLP conditions in mice, dosed by gavage with 500, 1000, and 2000 mg/kg bodyweight. The salt imidazole hydrochloride dissociates into protonated imidazole and chloride in the stomach following oral gavage and did not induce micronuclei at any dose or any harvesting time, which were set at 16, 24, and 48 hrs after dosing. The animals showed signs of toxicity at 500 mg/kg bodyweight and above. The number of polychromatic and normochromatic erythrocytes was not statistical significantly different from the control, therefore it may be concluded, that imidazole was not toxic to the bone marrow. Imidazol was found to be not clastogenic or aneugenic in this test (BASF AG, 1993).

Conclusion

Imidazole was not mutagenic in bacterial test systems generally meeting OECD TG 471 with the *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, or TA 1537, with or without the presence of metabolic activation by S-9 mix containing rat liver microsomes, with or without preincubation. Imidazol did not induce Unscheduled DNA Synthesis in rat primary hepatocytes in a study equivalent to the OECD TG 482. It was not clastogenic in the mouse micronucleus test according to the OECD TG 474 when imidazole hydrochloride was tested in vivo. The salt dissociates into protonated imidazole and chloride in the stomach following oral gavage.

3.1.7 Carcinogenicity

No studies concerning the long-term toxicity and/or carcinogenic potential of imidazole are available.

Imidazole failed to induce mammalian cell transformation when tested in a study using mouse fibroblasts. The test substance concentrations (0.1, 1, 2, 4 mg/ml) reached the cytotoxic concentration range of approx. 2 mg/ml (Forster et al., 1992).

Conclusion

No studies concerning the long-term toxicity and/or carcinogenic potential of imidazole are available. It is, however, mentioned that imidazole was negative in the mouse fibroblast cell transformation test.

3.1.8 Toxicity for Reproduction

Studies in Animals

Effects on Fertility

One- or two-generation studies are not available. However, a thorough histopathological examination of all male and female reproductive organs was performed in the 90-d repeated toxicity study according to OECD TG 408. Examination of sperm quality parameters in testes and epididymides were included, i.e. sperm count, sperm motility and sperm morphology were examined. Wistar rats (5 m/5 f per sex and dose group) were dosed with 0, 20, 60, 180 mg imidazole/kg bw per day via gavage and sacrificed on day 92. No changes of the male and female reproductive organs including sperm quality were noted in any of the dose groups up to and including 180 mg/kg bw per day. For further detail on general toxicity see chapter 3.1.5 (BASF AG, 2002g).

Conclusion

No reproductive toxicity studies are available. However, no changes of the male and female reproductive organs including sperm quality were noted in a rat subchronic 3-months study according to OECD TG 408. Imidazole was given by gavage at 20, 60, and 180 mg/kg bodyweight per day. The NOAEL for these endpoints was 180 mg/kg bw per day.

Developmental Toxicity

Imidazole was tested in a rat study conducted under GLP in accordance with OECD 414 at dose levels of 20, 60, and 180 mg/kg bw per day. It was administered to pregnant rats on days 6 through 19 of gestation (BASF AG, 2002h).

No signs of maternal toxicity, fetal or developmental toxicity were noted at 20 and 60 mg/kg bw per day. At 180 mg/kg bw per day significantly reduced food intake (-13 %) was noted when the treatment was started. This was reflected by a statistical significantly reduced body weight gain on gestational days 6 - 8 (-45 %) and 17 - 20 (-34 %). However, terminal body weight was comparable in all groups, and corrected terminal body weight gain was also comparable in all groups. The effect on body weight gain on gestational days 17 - 20 is due to a significant decrease of the gravid uterus weight (-26 %), high rate of resorptions (see below) and distinctly lower mean fetal body weight (see below), rather than maternal toxicity. The number of live fetuses per litter was significantly reduced and the postimplantation loss was 43 % compared to only 8 % in the control being statistically significant.

Examination of the live fetuses from high dose dams revealed no changes with respect to sex distribution. The mean fetal body weight was reduced by 14 %. Further, the incidence of external malformations (anasarca and/or cleft palate) was significantly increased. About 10 % of the high dose fetuses were affected (13/132 fetuses; in 7/22 litters) while no such changes were observed in the control.

Skeletal malformations were also statistically significantly increased: 7.8 % affected fetuses per litter (7/73 fetuses in 5/21 litters) were noted in the high dose group compared to 1.1 % in the control. The incidences of shortened scapula, bent radius, bent ulna, malpositioned and bipartite sternebrae were statistically significantly increased Soft tissue variations (dilated renal pelvis and ureter) were significantly increased in fetuses from high dose dams compared to controls (27 % vs. 6.4 %).

The incidences of skeletal variations, mainly delays of the ossification process, were statistically significantly increased from 91 % in the control group to 98.4 % in the high dose group. In historical control animals the mean occurrence of skeletal variations is 92.6 % (range 87.0 - 98.1 %).

Some indication of developmental toxicity was obtained in a whole embryo culture test when rat and mouse embryos were exposed to imidazole at 30 and 60 µg/ml in vitro. The findings of this teratogenicity screen included reduced yolk sac diameter and crown rump length, and decreased brain size observed in up to 100 % of treated embryos. Mortality was up to 83 % in this exploratory study (Daston et al., 1989).

Conclusion

In a study conducted in accordance to OECD TG 414 imidazole was developmental toxic and teratogenic at a dose of 180 mg/kg bw per day showing some maternal toxic effects which is not likely to be the sole cause of the teratogenic effect. The incidence of external and skeletal malformations was significantly increased up to 10 %. Furthermore there were soft tissue variations observed. The NOAEL was 60 mg/kg bw per day for maternal toxicity, developmental toxicity, and teratogenicity.

3.2 Initial Assessment for Human Health

Imidazole is readily absorbed and excreted in humans and in test animals after oral and rectal administration. Peak plasma levels are reached within 15 to 30 minutes in rats and within approx. 3 hours in humans. Elimination half-life in humans is approx. 2 to 3 hours. Therefore a potential for bioaccumulation is unlikely. Induction of microsomal P450 enzyme in the liver cells of rats and rabbits is restricted to certain isoenzymes such as 7-ethoxycoumarin-O-deethylase and isoenzyme 3a. However, no such induction was seen in Syrian golden hamster.

Imidazole is of moderate oral toxicity in a scientifically valid study. LD_{50} in rats was determined to be 960 - 970 mg/kg bw. 80 % Imidazole is corrosive to skin under occlusive conditions. Imidazole is irritating to rabbit eye when tested according to OECD TG 405. Persistent large size cornea opacity indicates the potential of severe eye injury after eye contact. No sensitization study is available.

Liver and kidney are target organs in subacute and subchronic (OECD TG 408) rat studies at dose levels of 180 mg/kg bw per day and above. Slight centrilobular liver cell hypertrophy and relative liver weight increase was noted. Diffuse ζ2u-microglobulin accumulation was noted in the proximal tubules of the renal cortex only in male rats but was considered to a species-specific effect. The NOAEL was approximately 60 mg/kg bw per day.

Red blood cells were additionally affected in 28-d experiments. Female rats receiving 125 mg/kg bw per day or more and male rats receiving 500 mg/kg bw per day were affected. The NOAEL was approximately 62.5 mg/kg bw per day. The effect on red blood cells was, however, not confirmed in the 90-day guideline study when rats received up to 180 mg/kg bw per dayImidazole was not mutagenic in bacterial test systems generally meeting OECD TG 471 with the *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, or TA 1537, with or without the presence of metabolic activation by S-9 mix containing rat liver microsomes, with or without preincubation. Imidazol did not induce Unscheduled DNA Synthesis in rat primary hepatocytes in a study equivalent to the OECD TG 482. It was not clastogenic in the mouse micronucleus test according to the OECD TG 474 when imidazole hydrochloride was tested in vivo. The salt dissociates into protonated imidazole and chloride in the stomach following oral gavage.

No reproductive toxicity studies are available. However, no changes of the male and female reproductive organs including sperm quality were noted in a rat subchronic 3-months study according to OECD TG 408. Imidazole was given by gavage at 20, 60, and 180 mg/kg bodyweight per day. The NOAEL for these endpoints was 180 mg/kg bw per day.

In a study conducted in accordance to OECD TG 414 imidazole was developmental toxic and teratogenic at a dose of 180 mg/kg bw per day showing some maternal toxic effects which is not likely to be the sole cause of the teratogenic effect. The incidence of external and skeletal malformations was significantly increased up to 10 %. Furthermore there were soft tissue variations observed. The NOAEL was 60 mg/kg bw per day for maternal toxicity, developmental toxicity, and teratogenicity.

No studies concerning the long-term toxicity and/or carcinogenic potential of imidazole are available. It is, however, mentioned that imidazole was negative in the mouse fibroblast cell transformation test.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

Fish

In a screening test with the Golden orfe *Leuciscus idus* 6 concentrations, from 250 -400 mg/l (nominal) plus control, were tested. An LC_{50} (48 h) of 284 mg/l (nominal) was calculated (BASF AG, 1977). With ECOSAR a 96h-LC50 for fish of 327 mg/l can be estimated.

Invertebrates

A test following Directive 79/831/EEC, C2 with *Daphnia magna* with 5 nominal concentrations ranging from 31.25 – 500 mg/l, resulted in an EC_{50} (48 h, immobilisation) of 341 mg/l (BASF AG, 1988c).

Algae

Acute Toxicity to *Scenedesmus subspicatus* was determined in a study, following DIN 38 412 part 9, with 6 nominal concentrations ranging from 5 – 250 mg/l. The E_rC_{50} for growth rate (72 h) was 133 mg/l and the NOEC 25 mg/l; corresponding values for the endpoint biomass were 127 mg/l and 10 mg/l respectively. After 96 h exposure the E_rC50 (96 h) was 130 mg/l and the E_bC50 (96 h) was 82 mg/l. (BASF AG, 1989c; BASF AG, 2002f).

Chronic Toxicity Test Results

No chronic aquatic toxicity data was available.

Toxicity to Microorganisms

In a respiration test using activated sludge according to OECD 209 with 5 nominal concentrations ranging from 9.6 – 1000 mg/l an EC_{50} (30 min) of > 1000 mg/l was obtained (BASF AG, 2003b).

Acute Toxicity to *Pseudomonas putida* was determined in a study, following DIN 38 412 part 8, with 7 nominal concentrations ranging from 312.5 – 10 000 mg/l. An EC_{50} (17 h) of 1175 mg/l was calculated (BASF AG, 1988d).

The toxicity of imidazole to *Rhizobium sp*. strains isolated from mung, urd and cowpea was determined in a growth inhibition test after an exposure period of 5 days. The EC_{100} was reported to be ca. 3000 mg/l (Gupta and Sud, 1972).

A bioassay with the free-living ciliate *Tetrahymena pyriformis* was conducted by Schultz and Cajina-Quezada (1982) and Schultz (1983). The concentration which elicited 50 % impairment of the population growth (IGC_{50}) was determined to be 680 mg/l after 48 h.

4.2 Terrestrial Effects

There are no data available on the terrestrial organisms.

4.3 Other Environmental Effects

There are no data available on other environmental effects.

4.4 Initial Assessment for the Environment

Distribution modelling predicts water to be the main target compartment for imidazole. The substance tends not to accumulate in biota (log K_{ow} = -0.02, measured). The calculated log K_{oc} of 0.99 suggests that adsorption to suspended solids is not to be expected. From the pKa-value of 14.9 it can be assumed that under environmental conditions the substance is available as cation. Therefore, binding of the substance to the matrix of soils with high capacities for cation exchange (e.g. clay) cannot be excluded. However, no data was available for ionic-ionic interactions in soil.

Imidazole was readily biodegradable in a test conducted according to OECD 301 A with domestic sludge (98 % degradation after 18 days, 10d-window was fulfilled). Based on the chemical structure of the substance hydrolysis is not likely to occur. Imidazole entering the atmosphere is degraded by reaction with photo-chemically produced hydroxyl radicals with a half-life of 10.7 h.

The following aquatic effect concentrations are available:

Leuciscus idus LC_{50} (48 h) = 284 mg/l (nominal concentration)

Daphnia magna: EC_{50} (48 h) = 341 mg/l (nominal concentration)

Scenedesmus subspicatus: E_rC_{50} (72 h) = 133 mg/l, E_bC_{50} (72 h) = 127 mg/l, with a NOEC of 25 mg/l (growth rate) and 10 mg/l (biomass) (nominal concentration).

Although no analytical monitoring of the test substance concentration was performed and imidazole is subject to indirect photolysis in water, it is assumed that the effect values will not be < 100 mg/l. This assumption is based on the much lower concentration of OH radicals in the ecotoxicity tests compared to outdoor conditions and thus much lower half-lives than found in the photolysis studies described in section 2.2.

Using the aquatic toxic effect on the most sensitive species, *Scenedesmus subspicatus*, a $PNEC_{aqua}$ of 133 µg/l is derived by applying an assessment factor of 1000 according to the EU Technical Guidance Document.

5 RECOMMENDATIONS

Human Health:

The chemical possesses properties (corrosiveness to skin, irreversible damage to eyes, teratogenic effects) indicating a hazard for human health. Humans are exposed by consumer products (chemical concentrations up to 10 %) and at the workplace. Therefore, the chemical is a candidate for further work. An exposure assessment and if indicated a risk assessment is recommended.

Environment:

The chemical is currently of low priority for further work because of its low hazard potential.

6 REFERENCES

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Details of the literature search used
The data bases searched are indicated below.
Toxicology: Date of last literature search: April 09, 2003
JETOC
RTECS
AGRICOLA
CABA
CANCERLIT
TOXCENTER
TOXLINE
JICST-EPLUS
LIFESCI
TOXLIT
EMBASE
ESBIOBASE
EMBAL
HEALSAFE
CSNB
MEDLINE
IRIS
ATSDR TOX. PROFILES
ATSDR TOX: FAQS
CHEMFINDER
CIVS
GESTIS
GINC
NICNAS
NTP

Ecology: Date of last literature search: April 09, 2003
AQUASCI
BIOSIS
EMBASE
ESBIOBASE
LIFESCI
OCEAN
POLLUAB
SCISEARCH
TOXCENTER
TOXLINE
ULIDAT
DATALOG
CHEMFATE
BIODEG
AQUIRE
HSDB

I U C L I D

D a t a S e t

Existing Chemical ID: 288-32-4
CAS No. 288-32-4
EINECS Name imidazole
EC No. 206-019-2
Molecular Formula C3H4N2

Producer Related Part
Company: BASF AG
Creation date: 12-NOV-1992

Substance Related Part
Company: BASF AG
Creation date: 12-NOV-1992

Memo: master

Printing date: 14-JUN-2004
Revision date:
Date of last Update: **14-JUN-2004**

Number of Pages: **123**

Chapter (profile): Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, SIDS

1.0.1 Applicant and Company Information

Type: lead organisation
Name: BASF AG
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Flag: Critical study for SIDS endpoint
11-JUN-2002

1.0.2 Location of Production Site, Importer or Formulator

1.0.3 Identity of Recipients

1.0.4 Details on Category/Template

1.1.0 Substance Identification

Mol. Formula: C3 H4 N2
Mol. Weight: 68.08 g/mol

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002

1.1.1 General Substance Information

Purity type: typical for marketed substance
Substance type: organic
Physical status: solid
Purity: >= 99.5 - % w/w
Colour: colourless to yellow
Odour: amine-like

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (1)

1.1.2 Spectra

1.2 Synonyms and Tradenames

1,3-Diaza-2,4-cyclopentadiene

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

1,3-Diazole

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

1H-Imidazole (9CI)

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Glyoxalin

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Glyoxaline

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Imidazol

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Imidazole (8CI)

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Imutex

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Methanimidamide, N,N'-1,2-ethenediyl-

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Miazole

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002

1.3 Impurities

Purity type: typical for marketed substance
CAS-No: 693-98-1
EC-No: 211-765-7
EINECS-Name: 2-methylimidazole
Mol. Formula: C4 H6 N2
Contents: <= .3 - % w/w

Method: GC
Flag: non confidential, Critical study for SIDS endpoint
12-JUN-2002 (2)

Purity type: typical for marketed substance
CAS-No: 822-36-6

EC-No: 212-497-3
EINECS-Name: 4-methylimidazole
Mol. Formula: C4 H6 N2
Contents: <= .3 - % w/w

Method: GC
Flag: non confidential, Critical study for SIDS endpoint
12-JUN-2002 (2)

Purity type: typical for marketed substance
CAS-No: 20075-26-7
EC-No: 243-505-3
EINECS-Name: 1-(methoxymethyl)-1H-imidazole
Mol. Formula: C5 H8 N2 O
Contents: <= .3 - % w/w

Method: GC
Flag: non confidential, Critical study for SIDS endpoint
12-JUN-2002 (2)

Purity type: typical for marketed substance
EINECS-Name: high-boiling components
Contents: <= .3 - % w/w

Method: GC
Flag: non confidential, Critical study for SIDS endpoint
12-JUN-2002 (2)

Purity type: typical for marketed substance
CAS-No: 7732-18-5
EC-No: 231-791-2
EINECS-Name: water
Mol. Formula: H2 O
Contents: <= .2 - % w/w

Method: DIN 51777
Flag: non confidential, Critical study for SIDS endpoint
12-JUN-2002 (2)

1.4 Additives

1.5 Total Quantity

Remark: Production quantity:

world: 1.000 - 5.000 t/a (estimated for year 2002)
Flag: non confidential, Critical study for SIDS endpoint
25-APR-2003

1.6.1 Labelling

Labelling: provisionally by manufacturer/importer
Symbols: (C) corrosive
R-Phrases: (34) Causes burns
(22) Harmful if swallowed
S-Phrases: (22) Do not breathe dust

(36/37/39) Wear suitable protective clothing, gloves and eye/face protection
(26) In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
(45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (1)

1.6.2 Classification

Classified: provisionally by manufacturer/importer
Class of danger: corrosive
R-Phrases: (34) Causes burns

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (1)

Classified: provisionally by manufacturer/importer
Class of danger: harmful
R-Phrases: (22) Harmful if swallowed

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (1)

1.6.3 Packaging

1.7 Use Pattern

Type: type
Category: Non dispersive use

Flag: non confidential, Critical study for SIDS endpoint
10-MAR-1994

Type: industrial
Category: Chemical industry: used in synthesis

Flag: non confidential, Critical study for SIDS endpoint
10-MAR-1994

Type: use
Category: Intermediates

Remark: Intermediates for Herbicides and Pharmaceuticals

The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine, and nucleic acids. Therefore, the bulk of imidazole produced is used in the preparation of biologically active compounds.
Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (3)

Type: use
Category: other: hardener for epoxy resins

Flag: non confidential, Critical study for SIDS endpoint
25-APR-2003

Remark: Imidazole is used in the chemical industry as an intermediate in the production of pharmaceuticals, pesticides, dye intermediates, auxiliaries for textile dyeing and finishing, photographic chemicals and corrosion inhibitors.
Flag: non confidential, Critical study for SIDS endpoint
06-AUG-2003 (4)

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

Orig. of Subst.: Synthesis
Type: Production

Remark: Of the many known methods for producing imidazoles, only the following are of industrial importance:

The Radziszewski Reaction
In the generally applicable Radziszewski reaction, a 1,2-dicarbonyl compound is condensed with an aldehyde and ammonia (R1 = H) in a molar ratio of 1 : 1 : 2, respectively. Replacement of a molar equivalent of ammonia with a primary amine (R1 = alkyl or aryl) leads to the corresponding 1-substituted imidazoles.
The reaction is usually carried out in water or a water - alcohol mixture at 50 - 100 °C. Work-up may involve the usual processes (e.g., distillation, extraction, and crystallization). Distillation leads to imidazole with a purity > 99 %. The yield is generally 60 - 85 %. This process allows the most important simple imidazoles to be prepared from the staring materials listed in following table "Imidazole and Derivates - Table 3".

Dehydrogenation of D2-Imidazolines
D2-Imidazolines can be obtained by several routes from 1,2-diamino compounds and carboxylic acid derivatives:
1) by reaction of a diamine with a carboxylic acid over an acidic heterogeneous catalyst (e.g., alumina - phosphoric acid) in the gas phase;
2) by reaction of a diamine with a carboxylic acid nitrile in the presence of sulfur or copper salts in the liquid phase; or
3) by preparation of diformyl derivatives from a diamine and formic acid esters, followed by conversion to imidazoline in the gas phase at 200 - 350 °C over a heterogeneous catalyst (e.g., zinc oxide - alumina).
The D2-imidazoline is then dehydrogenated in the gas phase over a precious metal at ca. 300 °C or on alumina - zinc oxide at 400 - 500 °C. In some cases this synthesis gives even better yields than the Radziszewski reaction (e.g., for 2-aryl-substituted imidazoles).
Attached doc.: imidazol_table3.JPG
Flag: non confidential, Critical study for SIDS endpoint
11-SEP-2002 (3)

Orig. of Subst.: Synthesis
Type: Production

Remark: All manufacturers execute the production over Glyoxal. Only BASF as manufacturer is backwards-integrated.
Flag: non confidential, Critical study for SIDS endpoint
06-AUG-2003 (5)

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

Type of limit: MAK (DE)
Limit value: other: no MAK value available

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (6)

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

Classified by: other: VwVwS (Germany), Annex 2
Labelled by: other: VwVwS (Germany), Annex 2
Class of danger: 1 (weakly water polluting)

Flag: non confidential, Critical study for SIDS endpoint
30-AUG-2002 (7)

1.8.4 Major Accident Hazards

1.8.5 Air Pollution

1.8.6 Listings e.g. Chemical Inventories

Type: EINECS
Additional Info: EINECS No. 206-019-2

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (8)

Type: ENCS
Additional Info: ENCS No. 5-381

Remark: ENCS classification:
low molecular heterocyclic organic compounds
Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (8)

Type: ECL
Additional Info: ECL Serial No. KE-20937

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (8)

Type: other: SWISS
Additional Info: SWISS No. G-3311

Remark: SWISS classification:
Giftliste 1 (List of toxic substances 1), 31.May 1999
Toxic category 4: Acute oral lethal dose of 500 - 2000 mg/kg.
Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (8)

Type: TSCA

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (8)

Type: DSL

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (8)

Type: AICS

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (8)

Type: PICCS

Flag: non confidential, Critical study for SIDS endpoint
11-JUN-2002 (8)

1.9.1 Degradation/Transformation Products

1.9.2 Components

1.10 Source of Exposure

Remark: according to the Swiss, Danish and Swedish Products Registers, imidazole is contained in a large number of products, some of them may be available to consumers.

In 2001 in the Swedish Product Register there were 13 products on the Swedish market with a total amount of imidazole of 5 tons/a.

The Danish Product Register cited 18 products that contain imidazole in a total amount of 1 ton/a. 12 products have a content of imidazole of up to 2 %, 4 products a content of 2 - 20 %. Product types were process regulators and anti-freezing agents.

In the Swiss Product Register there were registered 51 products with 48 professional and 3 with consumer applications. Approx. 40 % of the products were used in photographic and laboratory applications by professional industry. Further applications were glues/adhesives, cement

filler or sealing compounds (approx. 10 %), paints, varnishes and lacquers (approx. 8 %), anti-freezing agents (approx. 8 %) and auxiliary and supplementary agents (approx. 8 %). Three (approx. 6 %) of the registered products were available to consumers as cleaning agents, washing agents and soap and special applications for swimming-baths.

In the Finnish Product Register 4 products containing imidazole in a content of 1 - 10 % are registered. The total quantity of imidazole in these products in the year 2001 was 1 ton. The most frequent use category reported was: photo chemical. The most frequent industrial uses reported are: publishing, printing and reproduction of recorded media.

Reliability: (4) not assignable
only short abstract available, original reference not available
Flag: Critical study for SIDS endpoint
14-JUN-2004 (9) (10) (11) (12)

Remark: No analytical monitoring of imidazole in the effluent of the waste water treatment plant of BASF AG takes place
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
14-JUN-2004 (13)

Remark: During production and internal processing at BASF AG, Ludwigshafen (Germany), less than 5 kg imidazole were emitted into the air in 2000.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
14-JUN-2004 (14)

1.11 Additional Remarks

Memo: Hazardous reactions:
Strong exothermic reaction with acids.
Dust exlosion hazard.

Flag: non confidential, Critical study for SIDS endpoint
15-MAY-2003 (1)

1.12 Last Literature Search

Type of Search: Internal and External
Chapters covered: 3, 4
Date of Search: 09-APR-2003

Flag: Critical study for SIDS endpoint
24-OCT-2003

Chapters covered: 1
Date of Search: 09-APR-2003

Flag: non confidential, Critical study for SIDS endpoint
24-OCT-2003

Chapters covered: 8

Date of Search: 09-APR-2003

Flag: non confidential, Critical study for SIDS endpoint
24-OCT-2003

Type of Search: Internal and External
Chapters covered: 2
Date of Search: 09-APR-2003

Flag: Critical study for SIDS endpoint
24-OCT-2003

Type of Search: Internal and External
Chapters covered: 5
Date of Search: 09-APR-2003

Flag: Critical study for SIDS endpoint
24-OCT-2003

Chapters covered: 5.10
Date of Search: 09-APR-2003

Flag: Critical study for SIDS endpoint
24-OCT-2003

Type of Search: Internal and External
Chapters covered: 5
Date of Search: 09-APR-2003

Flag: non confidential, Critical study for SIDS endpoint
08-APR-2004

1.13 Reviews

2.1 Melting Point

Value: = 88 - 90 degree C

Reliability: (4) not assignable
manufacturer/producer data without proof
25-MAY-2000 (1)

Value: 88.3 - 89.3 degree C
Decomposition: yes at > 120 degree C
Sublimation: no

Method: other: calorimetric determination comparable to OECD 102
Year: 1989
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: differential scanning calorimeter DSC-2 of Perkin-Elmer
Remark: reason for flagging this study: experimentally derived data
Test substance: imidazole, purity >= 99.74 mol%
Reliability: (2) valid with restrictions
study meets generally accepted scientific principles
Flag: Critical study for SIDS endpoint
12-SEP-2002 (15)

Value: = 89.8 degree C
Decomposition: no at degree C
Sublimation: no

Method: other: calorimetric determination comparable to OECD 102
Year: 1987
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: differential scanning calorimeter DSC-2 of Perkin-Elmer
Remark: reason for flagging this study: experimentally derived data
Test substance: imidazole, pure, > 99mol%
Reliability: (2) valid with restrictions
study meets generally accepted scientific principles
Flag: Critical study for SIDS endpoint
12-SEP-2002 (16)

Value: = 89.9 degree C
Decomposition: no at degree C
Sublimation: no

Method: other: calorimetric determination comparable to OECD 102
Year: 1991
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: differential scanning calorimeter DSC-2 and DSC-7 of
Perkin-Elmer
Remark: reason for flagging this study: experimentally derived data
Test substance: Imidazole, no further data.
Reliability: (2) valid with restrictions
study meets generally accepted scientific principles
Flag: Critical study for SIDS endpoint
12-SEP-2002 (17)

Value: 90 degree C

Method: other: no data

Reliability: (2) valid with restrictions
data from reliable handbook
30-AUG-2002 (3)

2.2 Boiling Point

Value: = 218.7 degree C at 1013.25 hPa
Decomposition: no

Method: other: comparable to OECD 104
Year: 1987
GLP: no

Method: the Normal Boiling Temperature was obtained by extrapolation from the vapour pressure curve.
Remark: measured temperature: range of 25 °C to 85.9 °C
Test substance: imidazole (solid), purity 99.5 %
Reliability: (2) valid with restrictions
study meets generally accepted scientific principles
30-AUG-2002 (18)

Value: = 256 degree C

Reliability: (4) not assignable
manufacturer/producer data without proof
25-MAY-2000 (1)

Value: = 256 degree C

Method: other: no data

Reliability: (2) valid with restrictions
data from reliable handbook
30-AUG-2002 (3)

Value: = 267.8 degree C at 1013.3 hPa
Decomposition: no

Method: other: comparable to OECD 104
Year: 1987
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: the Normal Boiling Temperature was obtained by extrapolation from the vapour pressure curve
Remark: measured temperature: range of 94.17 °C to 267.04 °C
reason for flagging this study: calculation based on experimentally derived data
Reliability: (2) valid with restrictions
scientifically acceptable method
Flag: Critical study for SIDS endpoint
30-AUG-2002 (19)

Value: = 268.1 degree C at 1013.25 hPa
Decomposition: no

Method: other: comparable to OECD 104
Year: 1987
GLP: no

Method: the Normal Boiling Temperature was obtained by extrapolation from the vapour pressure curve.
Remark: measured temperature: range of 101.18 °C to 267.7 °C
reason for flagging this study: experimentally derived data
Test substance: imidazole (liquid), purity 99.5 %
Reliability: (2) valid with restrictions
scientifically acceptable method
Flag: Critical study for SIDS endpoint
30-AUG-2002 (20)

2.3 Density

Type: density
Value: = 1.111 g/cm³ at 95 degree C

Method: other: comparable to OECD 109
Year: 1989
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: 25 cm3 glas pycnometer (multiple determination)
Remark: reason for flagging this study: experimentially derived data
Test substance: imidazole, purity 99.9 %
Reliability: (2) valid with restrictions
acceptable study, meets basic scientific principles
Flag: Critical study for SIDS endpoint
05-AUG-2003 (21)

Type: density
Value: 1.0257 at 110 degree C

Method: other:no data

Reliability: (2) valid with restrictions
data from reliable handbook
30-AUG-2002 (3)

Type: bulk density
Value: .55 - .63 g/cm³

Method: other: no data

Reliability: (2) valid with restrictions
data from reliable handbook
30-AUG-2002 (3)

Type: bulk density
Value: 470 - 570 kg/m3

Method: other: German Industrial Standard DIN 53468

Remark: the high variation of the bulk density max be due to its clotting properties
Reliability: (2) valid with restrictions
test procedure according to national standard with

restrictions (clotting properties of the chemical may cause high variation of the cited bulk density value)
30-AUG-2002 (22)

Type: bulk density
Value: = 500 - 600 kg/m3

Reliability: (4) not assignable
manufacturer/producer data without proof
25-MAY-2000 (1)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = .003 hPa at 20 degree C

Method: other (measured): Knudsen-effusion method
GLP: no data
Test substance: other TS: imidazole, purum grade

Reliability: (4) not assignable
manufacturer/producer data without proof
07-AUG-2003 (1)

Value: .0025 hPa at 24 degree C

Method: other (measured): Knudsen-effusion method

Remark: reason for flagging this publication: environmentally relevant temperature range tested
Result:

temperature (°C)	vapour pressure (Pa)
18.8	0.138
21.2	0.183
24.0	0.250
27.2	0.360
30.7	0.525
33.4	0.702
35.9	0.915

Reliability: (2) valid with restrictions
scientifically acceptable method and publication
01-DEC-2003 (23)

Value: = .00327 hPa at 25 degree C
Decomposition: no

Method: other (measured): comparable to OECD 104
Year: 1987
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Effusion method
Remark: reason for flagging this study: environmentally relevant temperature range tested
Result:

temperature (°C)	vapour pressure (hPa)
25.0	0.00327

	38.7 0.0125
	70.3 0.222
	85.8 0.756
	85.9 0.735
Test substance:	imidazole (solid), purity 99.5 %
Reliability:	(2) valid with restrictions
	study meets generally accepted scientific principles
Flag:	Critical study for SIDS endpoint
30-AUG-2002	(18)

Value:	= 1.284 hPa at 94.2 degree C
Decomposition:	no
Method:	other (measured): comparable to OECD 104
Year:	1987
GLP:	no
Test substance:	as prescribed by 1.1 - 1.4
Method:	dynamic with nitrogen
Result:	temperature (°C) vapour pressure (hPa)
	94.17 1.284
	101.48 2.001
	108.53 2.993
	124.52 6.976
	131.89 10.030
	156.30 29.935
	177.84 69.744
	187.89 100.250
	221.90 299.240
	252.76 697.370
	260.46 846.580
	267.04 995.980
Test substance:	imidazole, purity 99.88 area%
Reliability:	(2) valid with restrictions
	study meets generally accepted scientific principles
30-AUG-2002	(19)

Value:	= 2 hPa at 101.2 degree C
Decomposition:	no
Method:	other (measured): similar to OECD 104
Year:	1987
GLP:	no
Test substance:	as prescribed by 1.1 - 1.4
Method:	dynamic with argon
Result:	temperature (°C) vapour pressure (hPa)
	101.18 2.00
	108.34 3.00
	117.87 5.00
	124.47 7.00
	131.77 10.00
	146.90 20.00
	156.40 30.00
	169.13 50.00
	178.00 70.00
	187.93 100.00
	208.7 200.0
	240.2 500.0
	253.1 700.0
	267.7 1000.0

Test substance: imidazole, purity 99.5 %
Reliability: (2) valid with restrictions
study meets generally accepted scientific principles
24-MAY-2002 (20)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = -1.319

Method: other (calculated): Increment method by Rekker with the computer programme pro-logP of CompuDrug Ltd.
Year: 1989

Reliability: (2) valid with restrictions
scientifically acceptable method
24-MAY-2002 (24)

Partition Coeff.: octanol-water
log Pow: -.08

Reliability: (2) valid with restrictions
data from reliable handbook
06-OCT-2003 (25)

Partition Coeff.: octanol-water
log Pow: = -.02 at 25 degree C

Method: other (measured): comparable to OECD 107
Year: 1988
GLP: no

Method: Shake flask method
Remark: reason for flagging this study: acceptable study, which meets basic scientific principles
Test substance: imidazole, purity 99.9 %
Reliability: (2) valid with restrictions
study meets generally accepted scientific principles
Flag: Critical study for SIDS endpoint
30-AUG-2002 (26)

Partition Coeff.: octanol-water
log Pow: = .059

Method: other (calculated): Log Kow v1.66

Remark: experimental database (Log Kow v1.66) structure match: log Pow: -0.08 (reference: Hansch, C et al., 1995)
Reliability: (2) valid with restrictions
scientifically acceptable method
30-AUG-2002 (27)

Partition Coeff.: octanol-water
log Pow: = .06

Method: other (calculated)

Remark: log Pow = -0.08/0.04 obs., secondary quotation
Reliability: (2) valid with restrictions
scientifically acceptable method

24-MAY-2002 (28)

Partition Coeff.: octanol-water
log Pow: = .1

Method: other (calculated)

Reliability: (2) valid with restrictions
scientifically acceptable method
11-APR-2003 (29)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 633 g/l at 20 degree C
pH value: 10.5
Conc.: 67 g/l at 20 degree C

Reliability: (4) not assignable
manufacturer/producer data without proof
08-MAY-2002 (1)

Solubility in: Water
Value: = 66.3 other: g/100g solution at 20 degree C
pH value: = 10.5
Conc.: 68 other: g/l solution at 20 degree C

Remark: reason for flagging this endpoint: only experimentally derived data available on this enpoint
Result: solubility in water: 663 g/L
Test substance: imidazole, purity > 99.5 %
Reliability: (2) valid with restrictions
study in accordance with an internal standard method, but without detailed documentation.
Flag: Critical study for SIDS endpoint
15-MAY-2002 (22)

Solubility in: Water
Value: 241 other: g/100g solvent at 20 degree C

Method: other: no data

Reliability: (2) valid with restrictions
data from reliable handbook
30-AUG-2002 (3)

2.6.2 Surface Tension

2.7 Flash Point

Value: > 135 degree C

Remark: ignitable gases/vapours
Reliability: (4) not assignable
manufacturer/producer data without proof
25-MAY-2000 (1)

Value: 154 degree C

Method: other: no data

Remark: reason for flagging this information: secondary data from peer reviewed handbook
Reliability: (2) valid with restrictions
data from reliable handbook
Flag: Critical study for SIDS endpoint
30-AUG-2002 (3)

Method: other: internal BASF-Standard
GLP: no

Remark: > ca. 135 Grad C ignitable vapours
reason for flagging this parameter: experimentally derived data
Reliability: (2) valid with restrictions
test procedure according to national standard, without detailed documentation
Flag: Critical study for SIDS endpoint
11-APR-2003 (30)

2.8 Auto Flammability

Value: 480 degree C

Method: other: Gernam Industrial Standard DIN 51 794
GLP: no

Remark: Ignition temperature
reason for flagging this parameter: experimentally derived data
Reliability: (2) valid with restrictions
test procedure according to national standard, without detailed documentation
Flag: Critical study for SIDS endpoint
11-APR-2003 (30) (1)

2.9 Flammability

Method: other: internal BASF-Standard
GLP: no

Remark: reason for flagging this parameter: only data available on this endpoint
Result: not highly flammable (VDI 2263, part 1, 1.2)
Reliability: (2) valid with restrictions
test procedure according to national standard, without detailed documentation
Flag: Critical study for SIDS endpoint
11-APR-2003 (30)

2.10 Explosive Properties

Method: other: Sprengstoffgesetz v. 25.08.1969
GLP: no

Remark: reason for flagging this parameter: only data available on this endpoint
Result: not explosive (German explosives law)
Reliability: (2) valid with restrictions
test procedure according to national standard, without detailed documentation
Flag: Critical study for SIDS endpoint
11-APR-2003 (30)

2.11 Oxidizing Properties

Result: no oxidizing properties

Remark: due to the chemical structure
reason for flagging this parameter: only data available on this endpoint
Reliability: (2) valid with restrictions
Expert judgement
Flag: Critical study for SIDS endpoint
16-JUN-2000 (31)

2.12 Dissociation Constant

Acid-base Const.: pKa=14.9

Method: other: no data

Remark: reason for flagging this parameter: important data on this endpoint
Reliability: (2) valid with restrictions
data from reliable handbook
Flag: Critical study for SIDS endpoint
12-SEP-2002 (3)

Acid-base Const.: pKb=7.0

Method: other: no data

Remark: imidazole is not ionised significantly between the two pKa values. The pKa of ca. 7 represents gaining a hydrogen ion (basicity), and the pKa of ca. 14 represents loss of hydrogen (acidity)
reason for flagging this parameter: important data on this endpoint
Reliability: (2) valid with restrictions
data from reliable handbook
Flag: Critical study for SIDS endpoint
12-SEP-2002 (3)

Result: - pK1: approx. 7.15
temperature: at 25 °C
remark: total ionic strength I = 0.55

method: measurements of pH changes during titrations using glass electrodes
variation with temperature:

temperature (°C)	pK1
20	7.27
30	7.08
35	7.00
40	6.90

- pK2: 14.44 (uncertain)
temperature: 25 °C
remarks: estimated uncertainty is greater than 10 % of the value K
total ionic strength I = 0.5 (NaCl; calculated from hydrolytic date))
method: light absorbance measurements using solutions of alkalis of known concentrations
variation with temperature:

temperature (°C)	pK2
15.3	14.89
25.3	14.43
35.2	14.02

(themodynamic quantities are derived from the results)

- pK2: 14.17 (uncertain)
temperature: 25 °C
remarks: estimated uncertainty is greater than 10 % of the value K
in aqueous KOH, H_scale
method: light absorbance measurements using solutions of alkalis of known concentrations

Reliability: (2) valid with restrictions
data from reliable handbook
09-MAY-2003 (32)

Acid-base Const.: pka at 25 °C = 6.95

Method: other
GLP: no data

Reliability: (2) valid with restrictions
acceptable publication which meets basic scientific principles
07-AUG-2003 (33)

2.13 Viscosity

Test type: other
Test procedure: no data
Value: 2.696 mPa s (dynamic) at 100 degree C

Method: other: no data

Remark: reason for flagging this parameter: only data available on this endpoint
Reliability: (2) valid with restrictions
data from reliable handbook
Flag: Critical study for SIDS endpoint
30-AUG-2002 (3)

2.14 Additional Remarks

Memo: Appearance

Remark: reason for flagging this parameter: only data available on this endpoint
Result: Form: solid
Colour: colourless - yellow
Odour: amine-like
Reliability: (4) not assignable
manufacturer/producer data without proof

Flag: Critical study for SIDS endpoint
22-MAY-2002 (1)

Memo: Hazardous reactions

Remark: exothermic reaction with acids
dust explosion hazard
reason for flagging this parameter: only data available on this endpoint (exothermic reaction with acids)
Reliability: (4) not assignable
manufacturer/producer data without proof
Flag: Critical study for SIDS endpoint
22-MAY-2002 (1)

Remark: particle size: 7-60 µm
reason for flagging this parameter: only data available on this endpoint
Result: dust explosible
Reliability: (2) valid with restrictions
test procedure according to national standard, without detailed documentation
Flag: Critical study for SIDS endpoint
16-JUN-2000 (30)

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Rate constant: = .0000000000359 cm³/(molecule * sec)
Degradation: = 50 % after .4 day(s)

Method: other (calculated)

Test condition: 25 deg C
Reliability: (2) valid with restrictions
scientifically acceptable method
08-AUG-2003 (34) (35)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Rate constant: = .000000000036 cm³/(molecule * sec)
Degradation: = 50 % after 10.7 hour(s)

Method: other (calculated): AOP (v1.90)

Remark: calculation based on the following assumptions:
- 0.5E6 OH/cm3
- 24-h day
reason for flagging this calculation: model caluclation accepted by the US-EPA
Reliability: (2) valid with restrictions
scientifically acceptable method
Flag: Critical study for SIDS endpoint
08-AUG-2003 (27)

Type: water
INDIRECT PHOTOLYSIS
Sensitizer: OH
Degradation: = 50 % after 307 day(s)

Remark: pH = 6.8
rate constant: 8.7 x 10E9 l/mol*sec
[OH] = 3 x 10E-18 mol/l (entire water column (14m), annual means, [NO3- N] = 1.4 mg/l)
reason for flagging this study: important information on this endpoint
Reliability: (2) valid with restrictions
scientifically acceptable method and publication
Flag: Critical study for SIDS endpoint
08-AUG-2003 (36) (37)

Type: water
INDIRECT PHOTOLYSIS
Sensitizer: OH
Degradation: = 50 % after 4.4 hour(s)

Remark: pH = 6.8
rate constant: 8.7 x 10E9 l/mol*sec
[OH] = 5 x 10E-15 mol/l (summer, midday, shallow water body,

[NO3- N] = 14 mg/l)
reason for flagging this study: important information on this endpoint
Reliability: (2) valid with restrictions
scientifically acceptable method and publication
Flag: Critical study for SIDS endpoint
08-AUG-2003 (36) (37)

Type: water
INDIRECT PHOTOLYSIS
Sensitizer: OH
Degradation: = 50 % after 15.4 day(s)

Remark: pH = 6.8
rate constant: 8.7 x 10E9 l/mol*sec
[OH] = 6 x 10E-17 mol/l (mean value)
reason for flagging this study: important information on this endpoint
Reliability: (2) valid with restrictions
scientifically acceptable method and publication
Flag: Critical study for SIDS endpoint
08-AUG-2003 (38) (36)

Type: water
Light source: Sun light
INDIRECT PHOTOLYSIS
Sensitizer: other: singlet oxygen
Conc. of sens.: 24000000 molecule/cm³
Degradation: = 50 % after 32 hour(s)

Remark: pH = 7
rate constant : 150 000 000 l/(mol*sec)
reason for flagging this study: important information on this endpoint
Test condition: concentration of singlet oxygen in water: 4 x 10E-14 M
Reliability: (2) valid with restrictions
scientifically acceptable method and publication
Flag: Critical study for SIDS endpoint
08-AUG-2003 (39)

Type: water
INDIRECT PHOTOLYSIS
Sensitizer: other: singlet oxygen
Degradation: = 50 % after 4.8 hour(s)

Remark: rate of oxidation of imidazole by singlet oxygen oxidation:

K = 4x10E7 M-1*s-1
Test condition: concentration of singlet oxygen in aquatic systems: 10E-12 M
Reliability: (4) not assignable
secondary quotion
08-AUG-2003 (40)

3.1.2 Stability in Water

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Type: adsorption
Media: water - soil
Method: other: PCKOCWIN v1.66

Remark: reason for flagging this calculation: model calculation accepted by the US-EPA
the Koc of this structure may be sensitive to pH. The Koc may vary significantly with pH!
Result: Log Koc = 0.9879 (Koc = 9.724)
Reliability: (2) valid with restrictions
scientifically acceptable method
Flag: Critical study for SIDS endpoint
06-OCT-2003 (27)

Type: volatility
Media: water - air
Method: other: calculated: HENRYWIN v3.10

Remark: reason for flagging this calculation: model calculation accepted by the US-EPA
Result: Henry´s law constant: 3.76 E-006 atm*m3/mol or 0.38 Pa*m3/mol (at 25 °C)
Reliability: (2) valid with restrictions
scientifically acceptable method
Flag: Critical study for SIDS endpoint
06-OCT-2003 (27)

Type: volatility
Media: water - air
Method: other: calculated

Remark: reason for flagging this calculation: scientifically acceptable method, based on measured data
Result: Henry's law constant:

Input parameter:
vapour pressure: 0.327 Pa at 25 °C
water solubility: 663E3 g/m3 at 20 °C
molecular weight: 68.08 g/mol

H = vapour pressure * molecular weight / water solubility
H = 0.327 Pa * 68.08 g/mol / 663E3 g/m3
H = 3.4E-5 Pa * m3 * mol-1
Reliability: (2) valid with restrictions
scientifically acceptable method
Flag: Critical study for SIDS endpoint
04-AUG-2003 (41)

Type: other: volatilization from water
Media: water - air
Method: other: calculation

Remark: volatilization half life fom river : 5.4 days
volatilization half life from lake: 61.7 days
water depth: 1 m
wind velocity: 5 m/s for river, 0.5 m/s for lake
Current velocity: 1 m/s for river, 0.05 m/s for lake
Reliability: (2) valid with restrictions
scientifically acceptable method
06-OCT-2003 (27)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water
Method: Calculation according Mackay, Level I
Year: 1999

Method: Level I V 2.11 model
Remark: reason for flagging this calculation: only information available on this endpoint
the calculation is based on the following physical and chemical properties:
- molecular mass: 68.08 g/mol
- data temperature: 20 °C
- log Kow: -0.02
- water-solubility: 6.33E5 g/m3
- Henry´s law constant: 3.23E-5 Pa*m3/mol
- vapour pressure: 0.3 Pa
- melting point: 89.8 °C

-	Volume (m^3)	Density (kg/m^3)	org. C (g/g)	fish lipid (g/g)
Air	6.0E+09	1.185		
Water	7.0E+06	1000		
Soil	45000	1500	0.02	
Sediment	21000	1300	0.05	
susp. Sed.	35	1500	0.167	
Fish	7	1000		0.05
Aerosole	0.012	1500		

Result: the calculation is for the uncharged molecule:

over time the substance will distribute into the following compartments:
- water: 99.98 %
- soil: 0.0076 %
- sediment: 0.0076 %
- air: 0.001 %

Reliability: (2) valid with restrictions
scientifically acceptable method
Flag: Critical study for SIDS endpoint
06-OCT-2003 (27)

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, domestic
Concentration: 38 mg/l related to Test substance
20 mg/l related to DOC (Dissolved Organic Carbon)
Contact time: 18 day(s)

Degradation: 90 - 100 % after 18 day(s)
Result: readily biodegradable
Kinetic:

3 day(s)	< 0 %
5 day(s)	10 %
7 day(s)	96 %
14 day(s)	97 %
18 day(s)	98 %

Control Subst.: Aniline
Kinetic:

1 day(s)	2 %
5 day(s)	96 %

Method: OECD Guide-line 301 A (new version) "Ready Biodegradability: DOC Die Away Test"
Year: 1993
GLP: yes
Test substance: other TS: imidazole, purity: 99.76 %

Remark: reason for flagging this study: only ready test available
test device:
- 2 l Erlenmeyer flasks, liquid volume: 1000 ml

incubation:
- on a laboratory shaker, approx. 120 rpm
- temperature: 22 +- 2 °C

number of replicates:
- test substance (TS): 2
- reference substance (RS): 1
- blank (BC): 2
- inhibition control (IH): 1
- assay to examine physico chemical (abiotic) elimination (PC): 1
- adsorption control (AC): 1

inoculum:
- source: activated sludge, domestic (sludge from laboratory wastewater treatment plants fed with municipal sewage)

reference control:
- reference substance: aniline
- concentration: 20 mg/L related to DOC
- kinetic of reference substance:

1 day	2 % DOC-elimin.
3 day(s)	58 % DOC-elimin.
5 day(s)	96 % DOC-elimin.
14 day(s)	95 % DOC-elimin.
18 day(s)	99 % DOC-elimin.

inhibition control:
- substances: aniline + test substance
- concentration: aniline: 20 mg/L related to DOC
  test subst.: 20 mg/L related to DOC
- kinetic of inhibition control:

1 day(s)	1 % DOC-elimin.
3 day(s)	20 % DOC-elimin.
7 day(s)	70 % DOC-elimin.
14 day(s)	95 % DOC-elimin.
18 day(s)	97 % DOC-elimin.

assay to examine physico-chemical (abiotic) elimination:
- concentration: 20 mg/L related to DOC
- w/o inoculum, w mercury chloride to avoid microbial biodegradation
- kinetic of physico-chemical elimination:

1 day(s): 4 % DOC-elimin.
7 day(s): 1 % DOC-elimin.
14 day(s): 2 % DOC-elimin.
18 day(s): 5 % DOC-elimin.

adsorption control:
- concentration: 20 mg/L related to DOC
- w incoulum, w mercury chloride to avoid microbial biodegradation
- kinetic of adsorption control:
1 day (s): -2 % DOC-elimin.
5 day (s): -5 % DOC-elimin.

Result: - duration of the adaption phase (lag-phase): 5 days
- duration of the degradation phase (log-phase): 2 days
- physico-chemical (abiotic) elimination assay: <10 % (DOC) at the end of the test
- adsorption control: < 10 % (DOC) after 5 days
- inhibition assay: 97 % (DOC) after 18 days

Reliability: (1) valid without restriction guideline study

Flag: Critical study for SIDS endpoint

07-OCT-2003 (42)

Type: aerobic
Inoculum: activated sludge, industrial
Concentration: 398 mg/l related to DOC (Dissolved Organic Carbon)
Contact time: 8 day(s)
Degradation: = 83 % after 8 day(s)
Result: other: easily eliminated from water
Kinetic: 3 hour(s) = 11 %
1 day(s) = 2 %
4 day(s) = 34 %
6 day(s) = 62 %
8 day(s) = 83 %

Method: other: following OECD 302 B (Modified Zahn Wellens Test)
GLP: no
Test substance: other TS: Imidazole, 1 % aqueous solution (DOC 4810 mg/L)

Remark: test conditions:

- numer of replicates: test substance: 1 flask; abiotic physico-chemical elimination (evaporation control): 1 flask

- blank: no blank flask was tested in parallel, but a statistically obtained mean value of 17 mg/L DOC was used to calculate the elimination

- reference substance: no reference substance was tested in parallel

- inoculum: activated sludge, industrial (BASF wwtp, Ludwigshafen, Germany), final concentration in the test: 1 g/l dry weight

- initial test substance concentration in the test substance flask: 419 mg/L DOC (w/o inoculum; measured); 398 mg/L DOC (corrected for the inoculum)

Result: - elimination (%) and pH

time (days)	DOC (mg/L) blank	TS	PC	DOC eliminaiton (%) TS	PC	pH TS
0	17	390	390	6	0	7
0.125	17	370		11		7
1	17	407	400	2	-3	7,5
4	17	280		34		8
6	17	170	390	62	0	8
8	17	85		83		8

TS = test substance, PC = abiotic physico-chemical elimination (evaporation)

- the sigmoidal shape of the elimination curve is a strong indication of biodegradation

Reliability: (2) valid with restrictions
comparable to guideline study with acceptable restrictions

06-OCT-2003 (43)

Type: aerobic
Inoculum: activated sludge, industrial, adapted
Concentration: 400 mg/l related to DOC (Dissolved Organic Carbon)
Contact time: 8
Degradation: = 97 % after 8 day(s)
Result: other: easily eliminated from water

Method: other: following OECD 302 B (Modified Zahn Wellens-Test)
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: test conditions:

- numer of replicates: test substance: 2 flasks; blank: 1 flask

- reference substance: no reference substance was tested in parallel

- inoculum: adapted industrial activated sludge; source: industrial activated sludge from BASF wwtp was incubated in a Modidified Zahn Wellens Test in the presence of imidazole for 15 days. Then the adapted sludge was separated from the liquid and was used to perform a new Modified Zahn Wellens Test) with imidazole as the sole carbon source. Final concentration in the test: 1 g/l dry weight

- incubation: at room temperature (20-25 °C) on a magnetic stirrer, airated with air (sparging)

Result: - kinetic of elimination:

time (days)	TS1 (% DOC)	TS2 (% DOC)
0		
3 (*)	0	0
1	4	1
4	74	91
6	97	92
8	97	97

(*) hours; TS1/2 = test substance assay #1 and #2

- the sigmoidal shape of the elimination curve is a strong indication of biodegradation

Reliability: (2) valid with restrictions
comparable to guideline study with acceptable restrictions
06-OCT-2003 (44)

Type: aerobic
Inoculum: activated sludge, industrial, non-adapted
Concentration: 400 mg/l
Degradation: = 70 - 80 % after 15 day(s)
Result: other: easily eliminated from water

Method: other: following OECD 302 B (Modified Zahn Wellens-Test)
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: test conditions:

- numer of replicates: test substance: 2 flasks; blank: 1 flask

- reference substance: no reference substance was tested in parallel

- inoculum: industrial activated sludge; source: industrial activated sludge from BASF wwtp. Final concentration in the test: 1 g/l dry weight

- incubation: at room temperature (20-25 °C) on a magnetic stirrer, airated with air (sparging)

Result: - kinetic of elimination:

time (days)	TS1 (% DOC)	TS2 (% DOC)
0		
3 (*)	4	4
1	8	2
3	6	4
6	5	2
8	22	20
10	10	8
14	8	2
15	80	7

(*) hours; TS1/2 = test substance assay #1 and #2

Reliability: (2) valid with restrictions
comparable to guideline study with acceptable restrictions
06-OCT-2003 (45)

Type: aerobic
Inoculum: activated sludge, industrial
Degradation: ca. 88 % after 19 day(s)
Result: other: easily eliminated from water
Kinetic:
9 day(s) = 6 %
11 day(s) = 6 %
12 day(s) = 86 %
19 day(s) = 88 %

Method: other: following OECD 303 A (OECD Confirmatory Test)
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: mean HRT: 3 h; substance concentration in the feed: 50 mg/L DOC (theoretical value), substance addition: starting on day 9
Result: 80-90 % DOC-elimination between day 12 and day 19
Reliability: (2) valid with restrictions
Discrepancy between documented test parameters and standard methods, but scientifically acceptable
06-OCT-2003 (46)

Type: aerobic
Inoculum: activated sludge, adapted

Remark: Imidazole required the adaption of the activated sludge inoculum.
The biodegradability depends on the number of nitrogen atoms in the heterocyclic nucleus of the molecule. The higher the N-atom number the lower is the susceptibility to biological attack.
Reliability: (4) not assignable
not assignable (foreign language, only short english abstract available)
07-AUG-2003 (47)

3.6 BOD5, COD or BOD5/COD Ratio

B O D 5

Method: other
BOD5: = 4 mg/l
Method: other
Year:

R A T I O B O D 5 / C O D

BOD5/COD: = 0
Method:
Remark: COD =11600 mg/l
Result: biodegradation: < 1 % after 5 days
Test condition: 1% solution
Reliability: (4) not assignable
original reference not available
06-OCT-2003 (48)
Method:

C O D

Method: other: dichromate-method, Standard Method for the Examination of Water and Wastewater, APHA, US (1971)
Year:
COD: = 1430 mg/g substance
Method:
Remark: Measured COD is 93.5 % of the theoretical value of 1530 mg/g
Theoretical N as NH3: 50 %
Measured amount of N as NH3: 48.6 %

One Nitrogen is oxydized to NH3, the other Nitrogen is oxidized to N2
Reliability: (2) valid with restrictions
scientifically acceptable method and publication
07-AUG-2003 (49)

Method:
Year:
Method:
Remark: 582 compounds (among them imidazole) have been tested for their oxidizabilities. The methods using permanganate (in acid solution) and dichromate/H2SO4 (without and with Ag+ as catalyst) have been applied in accordance with standard procedures and legislative prescription respectively
Result: - theoretical COD: 1176.5 mg/g
(1882 mg/g)
- dichromate-COD according to DEV (+): 1276 mg/g
- dichromate-COD according to AEV (#): 1343 mg/g
- permanganate-COD 35 mg/g

(+) DEV = Deutsches Einheitsverfahren
(#) AEV = Amerikanisches Einheitsverfahren
Reliability: (2) valid with restrictions
scientifically acceptable method and publication
08-AUG-2003 (50)

3.7 Bioaccumulation

BCF: = 3.16

Method: other: calculation BCF Program (v2.14)

Remark: equation used to make BCF estimate: Log BCF = 0.50, log Kow used by BCF estimates: -0.08 (experimental)
Reliability: (2) valid with restrictions
calculated value in accordance with generally accepted standard methods
06-OCT-2003 (27)

BCF: = 3.16

Method: other: estimate according to Meylan W.M. et al., Environm. Toxicol. Chem., 18 (4), 664-672, 1999

Remark: - calculation based on the following assumption:
if log Kow <1 then log BCF = 0.5 (BCF = 3.16)

- log Kow used: log Kow -0.02 (source: BASF AG, Laboratory of Analytics, unpublished study, J.No. 130728/01, 15.09.1988)
Reliability: (2) valid with restrictions
scientifically acceptable method
27-JUN-2003 (51) (52)

3.8 Additional Remarks

Memo: Utilization of imidazole for growth by the purple non-sulfur bacterium Rhodopseudomonas species JA1

Method: Rhodopseudomonas species JA1 (isolate from Dairy effluent) was grown photoheterotrophically (anaerobic/light [2.400 lux] in fully filled reagent bottles (500 mL) on a modified Biebl and Pfennig´s medium(*). Either malate, succinate or pyruvate was used as sole carbon source for the bacterium.

Ammonium chloride served as nitrogen source in the above medium and the culture was incubated at 30 °C +-2 °C under light (2.400 lux) and anaerobic conditions.

Logarithmically growing cultures of the purple non-sulfur bacterium were inoculated into an assay medium containing basal salts and imidazole (1 mM; purity: 99 %) as sole carbon source in fully filled screw test tubes and incubated phototrophically (anaerobic, light 2.400 lux).

Growth was measured turbidometrically at 660 nm every day until two consistent optical values were observed.

Disapearance of imidazole was studied after 15 d in the culture supernatant after centrifuging (10000 rpm for 15´) the stationary phase culture of Rhodpseudomonas sp JA1. The initial and final absorption spectra of imidazole were analyzed and the difference in the optical density values at the absorption maximum was considered for calculating the disappearance of the compound.

(*) Biebl H., Pfennig N. (1981) Isolation of members of the family Rhodospirillaciae. In: Starr M.P. et al. (Eds), The Prokaryotes, 1, Springer-Verlag, New York. P 267

Result: Imidazole was used as sole "N"-source in the presence of an organic carbon compound (malate/succinate/pyruvate). Almost 100 % disappearance of the compound was observed from the culture supernatant. However, though disappearance of the compound was observed, the incorporation into the cell mass must not have occurred, since there was no increase in biomass when compared to that of control. Furthermore, there was no noticeable change in initial and final absorption sprectra, clearly indicating that there was no photobiotransformation. Under such conditions, the disappearance of the compound may presumably be explained as due to the passive absorption by the organism:
- Biomass yield of Rhodpseudomonas sp JA1 in the presence of 1 mM imidazole after 15 d: 1.76
- Biomass yield of Rhodpseudomonas sp JA1 without imidazole (= control) after 15 d: 1.96

Reliability: (2) valid with restrictions
acceptable publication which meets basic scientific principles

07-AUG-2003 (53)

Memo: expert judgement

Remark: When appropriate feeding of adopted biological SWTP is applied, no disturbance of the degradation activity of activated sludge is expected. The inhibition effect of the product was not tested. This statement is based on conclusions of analogy and the results of the degradation tests (Zahn-Wellens, OECD 302 B) and other information about the product.

Reliability: (4) not assignable
Expert judgement

24-OCT-2003 (54)

Memo: growth on imidazole by Mycobacterium aurum MO1

Method: Mycobacterium aurum was tested for its ability to degrade heterocyclic compounds (among them imidazole) analogous to morpholine to understand the mechanisms to open the morpholine carbon ring.
Experiments were carried out at 30 °C and pH 6.5 with 0.5 g/L of imidazole. The degradation was also studied in the presence of 0.1 g/L morpholine. Growth was followed by measuring OD550 nm. A positive profile of growth, with OD changing from 0.01 to 0.1-0.3 within 6 days, confirmed substrate degradation and metabolite formation.
Result: no growth was detected on imidazole
Reliability: (2) valid with restrictions
acceptable publication which meets basic scientific principles
07-AUG-2003 (55)

Memo: oxygen uptake

Method: - apparatus: respirometer
- medium: natural seawater: North Sea water fortified with Na2HPO4, NaNO3 and Triton X-100. pH was adjusted to 8.2
- imidazol-concentration: calculated on N: 3 mg N
Remark: it is known that nitrogen and phosphorus are major bacterial nutrients determining the growth rate of bacteria in oil-polluted seawater. The possibilities of fertilizing the oil-polluted area with suitable P- and N-compounds was investigated.
Result: - oxygen uptake: 6 mg (an oxygen uptake of around 100 mg or more shows that a N-containing compound is an acceptable nitrogen donor for oil biodegradation)
- evaluation: Imidazole turned out to be inactive
Reliability: (2) valid with restrictions
acceptable publication which meets basic scientific principles
14-APR-2003 (56)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: 283.6

Method: other: Screening-Test
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: - Test species: Leuciscus idus
- Body length: 6.2 (mean of 18 fish) cm
- Weight: 2.40 (mean of 18 fish) g
- Illumination: artificial light, day:night rhythm = 16:8
- Light intensity: about 80-200 Lux
- Pre-test: Duration: 48 h
- Main-test (1,2): Duration: 4h, 24h, 48h
- Control: Duration 48 h

Concentration (mg/L)	fish (No.)	No of dead fish after 4h	24h	48h	pH start	end
Pre-test						
1600	3		3	3	7	
1000	3		3	3	7	
500	3		2	3	7	
100	3	0	0	0	7	
10	3	0	0	0	7	
Main-test (1)						
400	5	0	5	5	7	
250	5	0	0	0	7	
160	5	0	0	0	7	
100	5	0	0	0	7	
Main-test (2)						
control	10		0	0	7	
400	10		10	10	7	7.2
364	10		10	10	7	
331	10		9	10	7	
302	10		6	8	7	
275	10		1	1	7	
250	10		1	3	7	7.2

- the effects are related to the nominal concentrations
reason for flagging this screening test: only reliable data available on this endpoint

Result: Probit analysis according to Finney (after 48 h) #:

LC5 (48 h) = 239.6 (216.485 - 262.773 mg/L)
LC50 (48 h) = 283.6 (271.431 - 295.825 mg/L)
LC95 (48 h) = 327.6 (302.624 - 352.6 mg/L) S= 96.14 %

Finney DJ, Probit Analysis (1971) Cambr Univ Press, 3rd edition

Reliability: (2) valid with restrictions
discrepancy between documented test parameters and standard methods, but scientifically acceptable
Flag: Critical study for SIDS endpoint
01-DEC-2003 (57)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 250
EC50: = 341.5
EC100: = 500

Method: other: Directive 79/831/EEC, Annex V, Part C
Year: 1984
GLP: no
Test substance: other TS: imidazole, purity: 99.8 %

Method: Procedures to determine EC-values after 48 h:
- EC50: Spearman-Kaerber
- EC0: highest concentration tested at which <= 10 % of the animals were immobile
- EC100: lowest concentration tested at which 100 % of the animals were immobile

(Sachs L (1974) Angewandte Statistik, Springer Verlag, Berlin, Heidelberg, New York, 4th edition)
Remark: reason for flagging this study: only reliable study available on this endpoint
Test conditions:
- Test water: reconstituted water using deionized water was prepared and then aerated (oil-free air) and stored for 24h hours to allow stabilization. The specifications at the start were: total hardness: 2.63 mmol/L, ratio Ca:Mg: 4:1, ratio Na:K: 10:1, conductivity: 615 µS/cm, pH: 7.9, acid capacity (Ks) up to pH 4.3: 0.79 mmol/L
- Solubility of imidazole in water: >500 mg/L at 21 °C
- Illumination: artifical light, day-night rhythm = 16:8 h
- Light intensity: about 1-8 µE/(m2*s) at a wave length of 400-750 nm
- Temperature: 20-22 °C (292-294 K)
- Test volume: 10 ml
- Test vessels: test tubes (glass) with flat bottom
- Replicates: 4 per concentration
- Volume/animal: 2 ml
- Number of animals/vessel: 5
- Total number of animals/conc.: 20
- Age of animals: 2-24 h
- Observation times: visually after 0, 3, 6, 24 and 48 h
- Observation parameters: swimming ability, pH, oxygen
- Test concentrations: 31.25, 62.5, 125, 250, 500, 0 (control) mg/L
- The effects are related to the nominal concentrations

Result: - Number of mobile test animals after exposure (48 h) to various test concentrations:

concentration (mg/L)	mobile daphnids
31.25	20

62.5	20
125.0	20
250.0	20
500.0	0
control	20

- Effect values after 48 h:
EC50 = 341.51 mg/L
95 % confidence limits: 319.08-365.51 mg/L

- Effect values after 24 h:
EC0 =500 mg/L
EC50 >500 mg/L
95 % confidence limits: no data
EC100 = >500 mg/L

- pH at start:

concentration (mg/L)	pH
31.25	8.32
62.5	8.44
125.0	8.59
250.0	8.75
500.0	8.75
control	8.28

- pH after 48 h:

concentration (mg/L)	pH
31.25	8.14
62.5	8.12
125.0	8.15
250.0	8.23
500.0	8.36
control	8.19

- Oxygen (O2, mg/L) at start:

concentration (mg/L)	oxygen
31.25	9.02
62.5	9.02
125.0	9.03
250.0	8.98
500.0	8.90
control	8.99

- Oxygen (O2, mg/L) after 48 h:

concentration (mg/L)	oxygen
31.25	8.2
62.5	8.13
125.0	8.15
250.0	8.16
500.0	8.13
control	8.16

Reliability: (2) valid with restrictions
comparable to guideline study with acceptable restrictions (exposure concentrations in the test and the stability of imidazole were not confirmed by analysis)
Flag: Critical study for SIDS endpoint
01-DEC-2003 (58)

Species: other: Culex pipiens quinquefasciatus (fourth instar larvae)
Unit: **Analytical monitoring:** no data

GLP: no data

Method: imidazole was dissolved in acetone. For determining the biological activity, fourth instar larvae were placed in tap water (pH 8-8.5) in 6-oz. wax paper cups. Aliquots of the aceton solution were added to the cups (3 replicates). The mortality was read 24 h after start of the exposure period. The cups were placed in a room with a temperature of 75-80 °F. Controls were run with the test
Remark: dosage response line was not determined
Result: LC50 >1.0 ppm (according to the authors: very low activity, since LC50 is above 0.5 ppm)
Reliability: (2) valid with restrictions
acceptable publication which meets basic scientific principles
30-JUN-2003 (59)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 25
LOEC: = 50
EC10: = 63.7
EC50: = 133

Method: other: German Industrial Standard DIN 38412, Part 9
GLP: no
Test substance: other TS: imidazole, purity: 99.8 %

Remark: reason for flagging this study: only reliable study available on this endpoint
test was performed according to the German Industrial Standard DIN 38412, Part 9:

- Species: Scenedesmus subspicatus, CHODAT, SAG 86.81
- Algae in test vessels at start: 12000-13000 cells/mL
- Test concentrations: 5, 10, 25, 50, 100, 250 mg/l
- Replicates: per concentration and control: 4; blank: 2 (w/o cells, per concentration)
- Control: uninoculated test medium at each concentration
- Tubes were incubated for 96 h at 24.8 Grad C
- Illumination: artifical light, permanent illumination
- Light intensitiy: about 60-120 µE/(m2*s) at a wave length of 400-700 nm
- Samples were taken at regular intervals (0, 24, 48, 72 and 96 h)
- Measurements: photometric determination (Pulse-fluorimeter, Chlorophyll fluorescence, spectral photometric scan: 300-780 nm); pH
- The EC values are calculated from the concentration-response relationship
- Statistics: Tallaria RJ, Jacob LS (1979) The Dose Response Relation in Pharmacology, Springer-Verlag, 98-10

- The effects are related to the nominal concentrations

Result: effect values based on biomass:
EbC10 (72 h) = 58.7 mg/L
EbC10 (96 h) = 33.6 mg/L
EbC50 (72 h) = 126.8 mg/L

EbC50 (96 h) = 81.8 mg/L
NOEC (72 h) = 10.0 mg/L
LOEC (72 h) = 25.0 mg/L

effect values based on growth rate:
EuC10 (96 h) = 20.0 mg/L
EuC50 (96 h) = 129.6 mg/L

- pH values at test start (w/o algae) and after 96 h (inoculated assays):

concentration (mg/L)	pH (0 h)	pH (96 h)
control	7.84	7.92
5	7.87	7.97
10	7.86	7.98
25	7.87	7.99
50	7.91	8.01
100	8.00	8.05
250	8.17	8.24

Reliability: (2) valid with restrictions
comparable to guideline study with acceptable restrictions (exposure concentrations in the test and the stability of imidazole were not confirmed by analysis)
Flag: Critical study for SIDS endpoint
01-DEC-2003 (60) (61)

Species: other algae: Gloeotaenium loitlesbergarium Hansgirg (freshwater green algae)
Endpoint: other: Inhibition of calcification
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
NOEC: 68.1
LOEC: 681

Method: other
GLP: no data

Method: species:
- Gloeotaenium loitlesbergarium Hansgirg. The freshwater alga exists in nature as a two- or four-celled colony.

general culture conditions:
- as described by Prasad and Chowdary, 1979
- calcium carbonate is deposited at the line of contact of the cells of the colony in the form of a band. These bands failed to develop when the alga was cultured on defined algal media such as Bold´s Basal Medium or Chu-10 medium. However, when cultured algae without bands were transferred to a medium containing 20 mg CaCl2, 0.5 mg Na2CO3 and 2 mg NaHCO§ per 100 ml dest. water, bands were formed as in nature. This medium, reffered to as induction medium, was used for inducing band formation (calcification) in the present study.

- stock solution of imidazole was prepared in the induction medium. Desired concentrations were obtained by dilution with induction medium. All the experiments were carried out in 100 ml Erlenmeyer flaks, each containing 40 ml induction medium, with or without inhibitior. Experiments were carried out in duplicate under continuous light of 2400 lx at 27 +-1°C.
- time of initiation of the band, the number of colonies

showing band initiation at that time, and the final percentage of banded colonies after 96 h were recorded. Observations were made from 10 random microscopic fields.

Result: effect of imidazole on band development:

imidazole (M)	initiation time (h)	colonies showing band initiation (%)
control	37	21
10-6	37	21
10-5	37	20
10-4	37	21
10-3	37	20
10-2	ni	0

ni = no initiation

imidazole (M)	banded colonies after 96 h (%)
control	88
10-6	88
10-5	88
10-4	87
10-3	87
10-2	0

Reliability: (2) valid with restrictions
acceptable publication which meets basic scientific principles

07-OCT-2003 (62) (63)

4.4 Toxicity to Microorganisms e.g. Bacteria

Species: Photobacterium phosphoreum (Bacteria)
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 231

Method: other: Microtox-Test
GLP: no
Test substance: other TS: imidazole; purity: 99 %

Remark: - species: luminescent marine bacterium Photobacterium phosphoreum

- effect value: measured concentrations at which there is a 50 % reduction of the light emission relative to controls at the stated exposure times

- unit: mg/l related to ppm

Result: EC50 after 5 min: -0.53 (log(L/mmol)
EC50 after 15 min: -0.48 (log(L/mmol)
EC50 after 30 min: -0.53 (log(L/mmol); 231 ppm

Reliability: (2) valid with restrictions
acceptable publication which meets basic scientific principles

04-AUG-2003 (64)

Type: aquatic
Species: Vibrio fisheri (Bacteria)
Exposure period: 5 minute(s)

Unit: mg/l **Analytical monitoring:** no
EC50: = 8910 - 12600

Method: other: ToxAlert 10, Microtox and LumiStox standard assays using the appropriate manufacturers's luminometer (see respective manual)
GLP: no
Test substance: no data

Result: EC50 (5 min): 11000 mg/l (ToxAlert10), 8910 mg/l (Microtox), and 12600 mg/l (Lumistox);
data related to nominal concentrations;
Test condition: temperature: 15 degree C, pH 7
reference standard: 440 mg/l Zinc sulphate in 2% NaCl

The same stock chemical solution was used to prepare the dilution series for each assay (ToxAlert 10, Microtox and Lumistox, respectively) and all tests were carried out by the same person on the same day.

test criterion: inhibition of light emitted by the bioluminescent bacteria Vibrio fischeri after 5 min compared with zero dose expressed as EC (effect concentration);
Reliability: (2) valid with restrictions
scientifically acceptable, commercially used assay systems
03-JUL-2003 (65)

Type: aquatic
Species: other bacteria
Unit: **Analytical monitoring:** no data

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Rhodobacter sphaeroides OU5 (ATCC 49885), Rhodopseudomonas palustris OU 11 (ATCC 51186) and Rhodopseudomonas species JA1 (isolate from Dairy effluent) were grown photoheterotrophically (anaerobic/light [2.400 lux] in fully filled reagent bottles (500 mL) on a modified Biebl and Pfennig´s medium(*). Either malate, succinate or pyruvate were used as sole carbon source for the species. Ammonium chloride served as nitrogen source in the above medium and the cultures were incubated at 30 °C +-2 °C under light (2.400 lux) and anaerobic conditions.

Logarithmically growing cultures of purple non-sulfur bacteria were inoculated into an assay medium containing basal salts and imidazole (1 mM; purity: 99 %) as sole carbon source in fully filled screw test tubes and incubated phototrophically (anaerobic, light 2.400 lux).

Growth was measured turbidometrically at 660 nm every day until two consistent optical values were observed.

Fifty percent growth inhibitiory concentration (IC50) and Minimum Inhibitory Concentration (MIC) were studied in a medium containing basal salts and malate and ammonium chloride as carbon and nitrogen source in fully filled screw cap test tubes.

(*) Biebl H., Pfennig N. (1981) Isolation of members of the family Rhodospirillaciae. In: Starr M.P. et al. (Eds), The Prokaryotes, 1, Springer-Verlag, New York. P 267

Result: A threshold of 10 mM was kept as an upper limit in assessing the toxicity of imidazole on purple non-sufur bacteria, because toxicity on microorganisms by N-substitutions of aromatic compounds fall within this range.
The organisms were not inhibited by imidazole, suggesting that this compound is not toxic to purple non-sulfur bacteria:

- Rb. sphaeroides OU5:	IC50 (mM)	>10
	MIC (mM)	>10
- Rb. palustris OU11:	IC50 (mM)	5.9
	MIC (mM)	>10
- Rhodopseudomonas sp JA1:	IC50 (mM)	>10
	MIC (mM)	>10

Reliability: (2) valid with restrictions
acceptable publication which meets basic scientific principles

30-JUN-2003 (53)

Type: aquatic
Species: other bacteria: activated sludge from laboratory waste water plant treating municipal sewage
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:** no
EC50: > 1000
EC20 : ca. 45
EC80 : > 1000

Method: OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year: 1993
GLP: yes
Test substance: other TS: imidazole, purity: 99.76 %

Method:
- incubation time:
  30 min
- temperature:
  20 +-2 °C
- test vessels:
  Erlemeyer-flasks (nominal volume: 250 ml)
- synthetic medium:
  8 ml/flask 100-fold concentrated OECD-medium
- oxygen concentration during aeration:
  >2.5 mg/L
- oxygen concentration immediately before measurement:
  >6.5 mg/L
- duration of the measurement of oxygen consumption:
  8-10 min
- reference substance:
  3,5-dichlorophenol
- reference substance concentration:
  100, 10, 1 mg/L
- test substance concentration:
  1000, 496, 248, 100, 9.6 mg/L (nominal)
- inoculum: concentration: 1 g/L dry substance; source: activated sludge from laboratory waste water plant treating municipal sewage

Remark: reason for flagging this study: only study available on this endpoint

Result:
- reference control:
  EC20 (30 min): ca. 4.0 mg/L
  EC50 (30 min): ca. 18 mg/L
  EC80 (30 min): ca. 70 mg/L

- reference control:

	oxygen consumption rate (mg O2/L*h)	% inhibition
inoculum blank:		
mean value	24	-
reference substance:		
1 mg/L	26	-8
10 mg/L	15	38
100 mg/L	3	88

- test substance:
  EC20 (30 min): ca. 45 mg/L
  EC50 (30 min): >1000 mg/L
  EC80 (30 min): >1000 mg/L

- test substance:

	oxygen consumption rate (mg O2/L*h)	% inhibition
inoculum blank:		
mean value	24	-
test substance:		
1000 mg/L	16	33
496 mg/L	17	29
248 mg/L	17	29
100 mg/L	17	29
9.6 mg/L	23	4

Reliability: (1) valid without restriction
guideline study, GLP

24-OCT-2003 (66)

Type: other: asparagin mannitol medium
Species: Rhizobium sp. (Bacteria)
Exposure period: 5 day(s)
Unit: mg/l **Analytical monitoring:** no data
EC100 : ca. 3000

Method: other: growth inhibition

Remark: reason for flagging this publication: important information on this endpoint
Rhizobium sp. strains isolated from mung, urd and cowpea

In case of concentrations of 0.3% of imidazol there was completely inhibitition of growth of Rhizobium strains, concentrations below 0.03% gave about 33% growth as compared to the control.

Reliability: (2) valid with restrictions
scientifically acceptable method
Flag: Critical study for SIDS endpoint

30-JUN-2003 (67)

Species: Pseudomonas putida (Bacteria)
Exposure period: 17 hour(s)

Unit: mg/l **Analytical monitoring:** no
EC10: = 722.6
EC50: = 1174.8
EC90 : = 2200.2

Method: other: German Industrial Standard DIN 38412, Part 8
GLP: no
Test substance: other TS: Imidazole, purity: 99.8 %

Remark: Pre-culture:
- Species: Pseudomonas putida, DSM 50026
- Incubated at 24 °C (297 K +- 1 K), 150 rpm for 7+-1 h
- Medium: AK-medium according to DIN 38412, Part 8 (draft)
- Test vessel: 300 ml-Erlenmeyer flasks, 1 baffle
- Liquid volume: 100 ml

Test conditions:
- Liquid volume: 10 ml
- Inoculum: 1 ml pre-culture (adjusted to 10 TE/F)
- Test medium: Ak-medium according to DIN 38412, Part 8 (draft)
- Test concentrations: 312.5, 625, 1250, 2500, 5000 and 10000 mg/L
- Replicates: inoculated: 4 per concentration and control; blank (w/o cells): 1 per concentration and control
- Incubated at 20°C (292 K), 150 rpm for 17 h
- Measurements: photometric determination at 436 nm and pH at test start and after 17 h
reason for flagging this study: most reliable data available on this endpoint

Result: - EC-values (17 h) are based on the nominal concentrations

- pH at the start (0 h; w/o cells) and after 17 h (w cells):

concentration (mg/L)	pH (0 h) (w/o cells)	pH (17 h) (w cells)
312.5	7.8	6.4
625	8.0	7.0
1250	8.3	7.3
2500	8.6	8.2
5000	8.9	8.4
7500	9.0	8.6
10000	9.1	8.8
control	6.9	4.9

Reliability: (1) valid without restriction
test procedure according to national standard
Flag: Critical study for SIDS endpoint
06-OCT-2003 (68)

Species: Tetrahymena pyriformis (Protozoa)
Unit: **Analytical monitoring:** no data

Method: other:Tetrahymena Bioassay (species: free-living ciliate Tetrahymena pyriformis)

Remark: reason for flagging this publication: important information on this endpoint
Result: IGC50 (48 h) = 680.1 mg/L (9.99 mmol/L)
(95 % confidence limits: 390.9-1183.6 mg/L [5.74-17.38 mmol/L])

(IGC50 = concentration which elicits 50 % impairment of population growth in 48-72 h)

Reliability: (2) valid with restrictions
acceptable publication which meets basic scientific principles

Flag: Critical study for SIDS endpoint

30-JUN-2003 (69) (70)

Species: Tetrahymena pyriformis (Protozoa)

Method: the assay used 250 ml Erlenmeyer flasks containing 50 ml of toxicant/proteose-peptone medium solution, and the method involed using graded concentration series evaluated in replicates. Population densities were estimated by absorbance at 540 nm. From these data, the 50 % population growth impairment concentration was determined by probit analysis

Result: ICG50 (48 h) = -1.000 (-log(mmol/L)

(IGC50 = concentration in -log (mmol/L), which elicits 50 % impairment of population growth in 48 h)

Reliability: (2) valid with restrictions
acceptable publication which meets basic scientific principles

30-JUN-2003 (71)

Species: other bacteria

Method: surface estuarine water and the California ground water were exposed to copper and imidazole and their complex (1:2 ratio), at varying concentrations (1, 10, 100 µM) for 17 h before measurements of bacterial heterotrophic activities started.
The effect of the compounds on bacterial heterotrophic activities was determined by measuring the mineralization rates (14CO2 production) of D-glucose.
14C-glucose (0.1 µg/L) was added to the water samples pre-exposed to copper and imidazole and incubated at 21 °C for 5 h.
pH of the California ground water (ca. pH 6.9) was adjusted to pH 4.6 and 8.4 to assess the pH effect on microbial activity

Result: - copper and imidazole enhanced bacterial heterotrophic activities (mineralization of glucose) at substrate concentrations below 1 µM in surface estuarine water

- effect of copper and imidazole on bacterial mineralization of glucose at different pH in the California ground water; values are expressed as % (SD) of added 14C-glucose mineralized:

treatment	pH		
	4.6	6.9	8.4
control	5.0(0.1)	7.3(0.3)	7.2(0.3)
+ copper (1 µm)	0(0)	2.3(0.1)	0.3(0)
+ copper (10 µm)	0(0)	0(0.2)	0.1(0)
+ copper (100 µm)	0(0)	0(0)	0 (0)
+ imidazole (1 µm)	1.2(0)	6.0(0.2)	3.0(0.1)
+ imidazole (10 µm)	0(0)	6.4(0.7)	2.6(0.5)
+ imidazole (100 µm)	0(0)	6.2(0)	3.2(0.2)

	+ copper/imidazole (1:2 µM)	0.2(0.2)	3.1(1.3)	0.3(0)
	+ copper/imidazole (10:20 µM)	0.1(0)	0.3(0.1)	0.1(0)
	+ copper/imidazole (100:200 µM)	0(0)	0.1(0)	0(0)

the toxicity of copper and imidazole seemed to increase as pH deviates from the natural pH (California ground water: pH 6.9).
Complexation with imidazole failed to relieve the inhibition by copper in all cases. Overall, acidic pH seemed to exert more influences on microbial metabolic activity in the ground water system.

Reliability: (2) valid with restrictions
acceptable publication which meets scientific principles

30-JUN-2003 (72)

Species: other bacteria

Remark: Enterotoxin specific activity of three strains of Clostridium perfrigens Type A was not increased by imidazole at concentrations of 0.01-10 mmol/l.
Incubation: at 37 Grad C for 8 and 12 h
Imidazole inhibits the sporulation of one of the strains

Reliability: (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

30-JUN-2003 (73)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

4.6.3 Toxicity to Soil Dwelling Organisms

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

5.0 Toxicokinetics, Metabolism and Distribution

In Vitro/in vivo:	In vivo
Type:	Toxicokinetics
Species:	rat
No. of animals, males:	5
Doses, males:	16.6 mg/kg bw
Vehicle:	water
Route of administration:	gavage

Test substance: other TS

Remark: Comparable albeit slightly higher imidazole plasma levels were found when rats were given the ITF 182 salt at a dose equimolar to the dose of imidazole:
After a single dose of ITF 182 at 0.24 mmol/kg bw (equivalent to 16.3 mg imidazole/kg bw):the mean plasma level of imidazole in the rat were as follows:
0.25 h: 0.15 mol/l (10.2 mg/l)
0.5 h: 0.14 mol/l (9.5 mg/l)
1 h: 0.10 mmol/l (6.8 mg/l)
2 h: 0.03 mmol/l (2.0 mg/l)
4 h: not detectable

Higher single doses of the salt led to imidazole plasma levels as follows:
ITF 182 at 0.48 mmol/kg bw (equivalent to 33.2 mg imidazole/kg bw):
0.25 h: 0.24 mol/l (16.3 mg/l)
0.5 h: 0.25 mol/l (17.0 mg/l)
1 h: 0.22 mmol/l (15.0 mg/l)
2 h: 0.13 mmol/l (8.9 mg/l)
4 h: not detectable
ITF 182 at 0.97 mmol/kg bw (equivalent to 33.2 mg imidazole/kg bw):
0.25 h: 0.30 mol/l (20.4 mg/l)
0.5 h: 0.39 mol/l (26.6 mg/l)
1 h: 0.31 mmol/l (21.1 mg/l)
2 h: 0.21 mmol/l (14.3 mg/l)
4 h: 0.18 mmol/l (12.3 mg/l)
8 h: not detectable

Result: After a single dose of 0.24 mmol/kg bw the mean plasma level in the rat were as follows:
0.25 h: 0.13 mol/l (8.8 mg/l)
0.5 h: 0.13 mol/l (8.8 mg/l)
1 h: 0.09 mmol/l (6.1 mg/l)
2 h: 0.03 mmol/l (2.0 mg/l)
4 h: not detectable

Test condition: TEST ANIMALS
Male fasted Wistar rats.
TS ADMINISTRATION

Single oral dose.
EXAMINATION
Imidazole was examined using HPLC. Minimal detectable amount was 0.02 mmol/l.

Test substance: Imidazole, unspecified, and ITF 182 (salt consisting of equimolar quantities of imidazole and 2-hydroxybenzoate)

Conclusion: In the rat imidazole is rapidly absorbed from the gut after oral administration. Peak plasma levels are reached within 15 minutes.
After equimolar doses of ITF 182, a salt consisting of 1:1 imdiazole:2-hydroxybenzoate, comparable plasma levels of imidazole were noted.
Therefore it may be assumed that the imidazole plasma levels observed after higher doses of ITF 182 may be used as a surrogate because they are possibly comparable with plasma levels that would have resulted from comparable oral doses of imidazole.
Reliability: (2) valid with restrictions
Exploratory study. Brief yet reliable description of application and results.
Flag: Critical study for SIDS endpoint
17-JUL-2003 (74)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: rat
No. of animals, males: 3
Doses, males: 3 µmol/kg bw (0.204 mg/kg)
Vehicle: physiol. saline
Route of administration: i.v.

Test substance: other TS

Result: Recovery of imidazole and metabolites in rat 24-h urine was as follows: imidazole 14%, hydantoin 39%, hydantoic acid 31%, others 4%.
No difference was noted in rats pretreated with 3-methylcholanthrene or phenobarbital. In contrast, rats pretreated with SKF525-A excreted 49% of the intact imidazole with urine.
Test condition: Rats were given imidazole by intravenous injection. Thereafter they were placed singly in metabolism cages for uncontaminated 24-h urine collection. Urine was subjected to Thin Layer chromatography. For quantitative examination of the identified metabolites (ninhydrin reaction) spots were removed into a vial for scintillation counting.

Autoradiography was used to examine distribution of radioactivity in tissues.
Test substance: [2-14C]-Imidazole, radiochemical purity >97%
Reliability: (3) invalid

Unsuitable test system: route of exposure not relevant.
13-JUL-2003 (75)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: human
No. of animals, males: 4
No. of animals, females: 6
Doses, males: 750 mg Selezen (containing 248 mg imidazole)
Doses, females: 750 mg Selezen (containing 248 mg imidazole)
Route of administration: other: oral and rectal

Test substance: other TS

Result: Pharmacokinetik parameters of imidazole (mean values):

		Tablet	Suppository
Cmax	(mg/l)	3.4	2.8
tmax	(min)	86	75
AUC	(mg h/l)	14.7	11.9
V	(l)	44.0	55.7
CL	(l/h)	20.0	23.7
t1/2ß	(h)	1.70	1.78
Ke	(1/h)	0.455	0.448
t1/2a	(h)	0.73	0.55
Ka	(1/h)	1391	1474

Legend:
Cmax maximum concentration (mg/l)
tmax time to reach Cmax (min)
AUC area under the concentration-time curve (mg h/l)
V distribution volume (l)
CL clearance from plasma (l/h)
t1/2ß elimination half-life (h)
Ke elimination constant (1/h)
t1/2a absorption half-life (h)
Ka absorption constant (1/h)

Bioavailability was calculated to be 85 (+/-7) %.

Test condition: TEST PERSONS
Mean age of was 36.1 (+/- 7.3) years, range between 26 and 50 years. Mean weight was 66.4 (+/- 13.5) kg.
ADMINISTRATION
Each subject received both the tablet and the suppository.
EXAMINATION
Blood samples were collected before administration and 30, 60, 90, 120, 240, 360, 480 min thereafter. Imidazole was determined using HPLC
EVALUATION
Kinetik parameters were calculated using an open one compartment model with a first order absorption rate using the least square method for non-linear regression. The general equation for the evaluation of the plasma concentration versus time is: Ct=-Ae(exp -Kat)+Be(exp-Ket) where Ct represents the plasma concentration at time t, and A and B are the ordinate at the origin.
Student's t-test for paired data was used to compare pharmacokinetic constants of imidazole and salicylic acid.

Test substance: ITF 182 (Selezen, antiinflammatory drug) containing equimolar quantities of imidazole and 2-hydroxybenzoate

Conclusion: Pharmacokinetic parameters are very similar after both oral or rectal administration. Absorption is rapid, maximum concentration is reached within approx. one hour.

Reliability: (2) valid with restrictions
Report meets accepted scientific standards and is described in sufficient detail.

Flag: Critical study for SIDS endpoint

17-JUL-2003 (76)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: human
No. of animals, males: 64
Doses, males: 750 mg of drug (containing 248 mg imidazole), or 3 times 750 mg drug/day for 3 -4 days (10

treatments)

Route of administration: other: oral tablet and/or drops

Half-lives: **1st** 3 hours **2nd 3rd**

Test substance: other TS

Result: Pharmacokinetic parameters of imidazole following oral dosing (tablet and drops combined, means):

	single dose	multiple dose
Cmax (mg/l)	3.59 (tablet)	2.87 (tablet)
Tmax (h)	0.79 (tablet)	1.04 (tablet)
t1/2ß (h)	2.98 +/- 1.13	1.86 +/- 0.78
protein binding (% of dose)	5 - 15%	
bioavailability	138%	113%

Legend:
Cmax maximum concentration (mg/l)
t1/2ß elimination half-life (h)

No statistically significant difference was noted between tablets and drops treatment, only one p-value was <0.01. Tolerability was good, no adverse reactions were noted. Topical application (gel 5%) did not show any systemic effects or adverse reactions. Local tolerability was reported to be very good.

Test condition: TEST PERSONS
4 groups of healthy male volunteers attended the study. Age ranged between 18-25 years. Body weight was within 20% of their ideal body weight.
ADMINISTRATION
Test persons received 1 tablet or 4o drops (750 mg drug in each case) in the morning after fasting overnight in the single dose study. In the crossover multiple dosing study they received 3 times one tablet (or 3 times 40 drops) for 3 days and one dosing in the morning of the 4th day.
In a pilot study topical application of a gel (5% drug) was studied with 4 test persons.
EXAMINATION
Medical, biochemical, and hematological examination was performed before and after the study. Blood samples were collected at 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 360, 480 min and 24 h after single dosing, and after 15, 30, 45, 60, 90, 120, 180, 240 min and 5, 6, 7, 8, 9, 10, 12 h, and thereafter every 12 hours. After the last dose on day 4 blood samples were taken at 0, 30, 60, 90, 120, 180, 240, 300, 360, min and 8, 24, 36 hours following the last dose.
Urine sampling periods were 0-2, 2-4, 4-6, 6-8, 8-24, 24-36 and 36-48 after single dosing. In the multiple dosing study periods were 0-12 and 12-24 h for days 1, 2, and 3; for day 4 periods were the same as after single dosing.
Clinical chemistry included bilirubin, transaminases, alcaline phosphatase, LDH, gamma-GT, triglycerides, cholesterol, creatinine, uric acid, glucose, total protein, albumine, globuline, sodium, potassium, chloride. hematology included red blood cell count, hemoglobin, hematocrit,

leucocytes including differential count, and platelets.
Imidazole was analyzed with HPLC.
EVALUATION
Kinetic parameters were calculated using an open one compartment model with a first order absorption rate using the least square method for non-linear regression. The general equation for the evaluation of the plasma concentration versus time is: Ct=-Ae(exp -Kat)+Be(exp-Ket) where Ct represents the plasma concentration at time t, and A and B are the ordinate at the origin.
Descriptive statistics included calculation of means, standard deviations, medians, minima and maxima. The means and the p-values between the application of imidazole and salicylic acid were calculated according to a paired Wilcoxon-test.

Test substance: ITF 182 (antiinflammatory drug) containing equimolar quantities of imidazole and 2-hydroxybenzoate

Conclusion: Pharmacokinetic parameters for imidazole are presented from single and multiple oral dose studies. No differences were noted between administration as tablet or as drops. Pharmacokinetic parameters were very similar in both the single and multiple dose study.

Imidazole is rapidly absorbed and excreted. Bioavailability is high, protein binding is low. It is concluded from these data that bioaccumulation is unlikely.

No adverse eeffects were noted after oral dosing, nor after topical application in a small number of volunteers as a gel (5%) in a dermal pilot study.

Reliability: (2) valid with restrictions
Meets accepted scientific standards and is described in sufficient detail

Flag: Critical study for SIDS endpoint

17-JUL-2003 (77)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
No. of Animals: 23
Vehicle: water
Doses: 500, 700, 1000, 1260, 2000, 4000, 5000 mg/kg bw
Value: ca. 970 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Although the animals have been observed for 7 days this does not invalidate the study as all mortalities occurred within one day.

Result: Signs of toxicity
Mortality (Dose in mg/kg bw: no. of animals that died/no. of animals):
5000: 1/1
4000: 1/1

2000: 1/1
1260: 5/5
1000: 2/5
700: 1/5
500: 0/5

During acute toxicity studies signs observed in both mice and rats included convulsions, disequilibria, lateral posture, death within one day.
Apathy, minor disequilibria, and accelerated respiration was noted in survivors.

Test condition: Test Animals
Rats (strain unspecified) were used.
Administration
The Test Substance was prepared in aqueous medium to give a final concentration of 10% imidazole, pH 9.
Evaluation
The LD50 was calculated on the Test Substance.
Test substance: Imidazole, degree of purity ca.100%
Reliability: (2) valid with restrictions
Study meets generally accepted scientific principles.
Observation period 7 days.
Flag: Critical study for SIDS endpoint
16-DEC-2003 (78)

Type: LD50
Species: rat
Vehicle: water
Doses: 500, 700, 1000, 1260, 2000, 4000, 5000 mg/kg bw
Value: ca. 960 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS

Remark: Although the animals have been observed for 7 days this does not invalidate the study as all mortalities occurred within one day.
Result: Signs of toxicity
Mortality (Dose in mg/kg bw: no of animals that died/no of animals):
5000: 1/1
4000: 1/1
2000: 5/5
1260: 4/5
1000: 3/5
700: 2/5
500: 0/5

During acute toxicity studies signs observed in both mice and rats included convulsions, disequilibria, lateral posture, death within one day.
Apathy, minor disequilibria, and accelerated respiration was noted in survivors.
Test condition: Test Animals
Rats (unspecified strain) were used.
Administration
The Test Substance was prepared in aqueous medium to give a final concentration of 10% imidazole, pH 9.

Evaluation
The LD50 was calculated on the Test Substance.
Test substance: Imidazole, technical, degree of purity ca. 95%
Reliability: (2) valid with restrictions
Study meets generally accepted scientific principles.

Flag: Critical study for SIDS endpoint
11-JUL-2003 (78)

Type: LD50
Species: rat
Value: 220 mg/kg bw

Method: other
GLP: no data
Test substance: other TS

Remark: The value cited in the secondary literature (220 mg/kg, in RTECS) cannot be confirmed since it was not found in the original literature. The publication exclusively deals with formaldehyde, glyoxal, and glutaraldehyde.
Test substance: Other. Imidazole was not tested
Reliability: (3) invalid
According to the original literature Imidazole was not tested. However, in order to be as transparent as possible the summary of the original publication is included.
16-DEC-2003 (79)

Type: LD50
Species: mouse
Value: 880 mg/kg bw

Method: other
GLP: no
Test substance: other TS

Test substance: Imidazole, unspecified
Reliability: (4) not assignable
Data from collection of data. Originally cited in patent information. No scientific data.
17-JUL-2003 (80)

Type: LD50
Species: mouse
Sex: male
No. of Animals: 30
Value: 1880 mg/kg bw

Method: other
GLP: no
Test substance: other TS: no data

Remark: Only males tested for LD50
Test condition: Minimum of 3 doses per test substance was administered to mice. 10 mice per dose level were used.
Test substance: Imidazole, unspecified. Further, substituted imidazoles were tested.
Reliability: (2) valid with restrictions
Scientific publication without detailed documentation.
17-JUL-2003 (81)

Type: LD50
Species: guinea pig
Value: = 760 mg/kg bw

Method: other: no details reported
GLP: no data
Test substance: other TS

Remark: The value cited in the secondary literature (760 mg/kg, in RTECS) cannot be confirmed since it was not found in the original literature. The publication exclusively deals with formaldehyde, glyoxal, and glutaraldehyde.
Test substance: Other. Imidazole was not tested
Reliability: (3) invalid
According to the original literature Imidazole was not tested. However, in order to be as transparent as possible the summary of the original publication is included.
16-DEC-2003 (79)

5.1.2 Acute Inhalation Toxicity

5.1.3 Acute Dermal Toxicity

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: rat
Route of admin.: i.p.
Value: 620 mg/kg bw

Test substance: other TS

Remark: Males tested for LD50
Test substance: Imidazole, unspecified
Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.
17-JUL-2003 (82)

Type: LD50
Species: mouse
Vehicle: water
Route of admin.: i.p.
Value: = 520 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS

Result: Signs of toxicity
During acute toxicity studies signs observed in both mice and rats included convulsions, disequilibria, lateral posture, death within one day at high and intermediate dose levels.
Apathy, minor disequilibria, and accelerated respiration was noted in survivors.
Test condition: Test Animals
Mice were used.
Administration

The Test Substance was prepared in aqueous medium to give a final concentration of 10% imidazole, pH 9.
Evaluation
The LD50 was calculated on the Test Substance.
Test substance: Imidazole, degree of purity ca. 100%
Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.
12-JUL-2003 (78)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: 300 mg/kg bw

Test substance: other TS

Test substance: Imidazole, unspecified
Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.
17-JUL-2003 (80)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: 610 mg/kg bw

Test substance: other TS

Remark: Males tested for LD50
Test substance: Imidazole, unspecified
Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.
17-JUL-2003 (81)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: 580 mg/kg bw

Test substance: other TS

Remark: Males tested for LD50
Test substance: Imidazole, unspecified
Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.
17-JUL-2003 (82)

Type: LD50
Species: mouse
Vehicle: water
Route of admin.: i.p.
Value: 500 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS

Result: Signs of toxicity
During acute toxicity studies signs observed in both mice and rats included convulsions, disequilibria, lateral posture, death within one day at high and intermediate dose

levels.

Apathy, minor disequilibria, and accelerated respiration was noted in survivors.

Test condition: Test Animals
Mice were used.
Administration
The Test Substance was prepared in aqueous medium to give a final concentration of 10% imidazole, pH 9.
Evaluation
The LD50 was calculated on the Test Substance.

Test substance: Imidazole, technical grade ca. 95%

Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.

12-JUL-2003 (78)

Type: LD50
Species: rat
Route of admin.: s.c.
Value: 626 mg/kg bw

Test substance: other TS

Test substance: Imidazole, unspecified

Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.

17-JUL-2003 (80)

Type: LD50
Species: mouse
Vehicle: water
Route of admin.: s.c.
Value: = 560 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Signs of toxicity
During acute toxicity studies signs observed in both mice and rats included convulsions, disequilibria, lateral posture, death within one day at high and intermediate dose levels.
Apathy, minor disequilibria, and accelerated respiration was noted in survivors. Necrosis was noted at the site of injection.

Test condition: Test Animals
Mice were used.
Administration
The Test Substance was prepared in aqueous medium to give a final concentration of 10% imidazole, pH 9.
Evaluation
The LD50 was calculated on the Test Substance.

Test substance: Imidazole, degree of purity ca. 100%

Reliability: (3) invalid

Unsuitable test system. Route of exposure not relevant.

06-JUL-2003 (78)

Type: LD50
Species: mouse

Route of admin.: s.c.
Value: 817 mg/kg bw

Test substance: other TS

Test substance: Imidazole, unspecified
Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.
17-JUL-2003 (80)

Type: LD50
Species: mouse
Vehicle: water
Route of admin.: s.c.
Value: 530 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS

Result: Signs of toxicity
During acute toxicity studies signs observed in both mice and rats included convulsions, disequilibria, lateral posture, death within one day at high and intermediate dose levels.
Apathy, minor disequilibria, and accelerated respiration was noted in survivors. Necrosis was noted at the site of injection.
Test condition: Test Animals
Mice were used.
Administration
The Test Substance was prepared in aqueous medium to give a final concentration of 10% imidazole, pH 9.
Evaluation
The LD50 was calculated on the Test Substance.
Test substance: Imidazole technical grade ca. 95%
Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.
12-JUL-2003 (78)

Type: LD50
Species: dog
Route of admin.: s.c.
Value: = 28 mg/kg bw

Method: other: no details reported
GLP: no data
Test substance: other TS

Remark: The value cited in the secondary literature (28 mg/kg, in RTECS) cannot be confirmed since it was not found in the original literature. The publication exclusively deals with formaldehyde, glyoxal, and glutaraldehyde.
Test substance: Other. No Imidazole tested.
Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.
No imidazole tested. However, in order to be as transparent as possible the summary is included.
16-DEC-2003 (79)

Type: LDLo

Species: cat
Route of admin.: s.c.
Value: 125 mg/kg bw

Test substance: other TS

Test substance: Imidazole, unspecified
Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.
17-JUL-2003 (80)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: 475 mg/kg bw

Test substance: other TS

Test substance: Imidazole, unspecified
Reliability: (3) invalid
Unsuitable test system. Route of exposure not relevant.
17-JUL-2003 (80)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration: 80 % active substance
Exposure: Occlusive
Exposure Time: 4 hour(s)
No. of Animals: 2
Vehicle: water
Result: corrosive

Method: other: BASF-Test
GLP: no
Test substance: other TS

Method: Similar to OECD 404; however occlusive exposure instead of semi-occlusive
Result: Skin reactions after 1-hr exposure time:
Mild erythema was seen in all (4/4) animals 1 hr after removal of the occlusive dressing. Erythema subsided in one animal overnight. Focal necrosis developed in the other 3 animals within 24 and 48 hr. Findings at the end of the observation period (8 d) were as follows:
2/4 animals showed no erythema but showed desquamation (1/4) and focal necrosis (1/4). Slight erythema, desquamation and extended focal necrosis was seen in the 3rd animal, and severe erythema with superficial necrosis was noted in the 4th animal.
Oedema were absent after patch removal but grade 2 oedema were noted in 2/4 and 3/4 animals after 24 and 48 hr, respectively. Oedema had resolved on day 8 of observation.

Skin reactions after 4-hr exposure time:
Well defined, moderate erythema and oedema were seen in both animals after removal of the occlusive dressing.

Erythema became severe overnight and rested severe until the end of the observation period. Soft necroses developed overnight in both animals. Leather-like necrosis indicative of full thickness necrosis was noted in both animals and confirmed by pathology after sacrifice of the animals. Oedema started to resolve overnight but rested as grade 2 until day 8.

General toxicity:
No signs noted after 1-hr or 4-hr exposure.

Test condition: TEST ORGANISMS
A total of 6 Wiener White rabbits, mean bodyweight ca. 2.7 kg, were used.

ADMINISTRATION
0.5 ml of paste with water containing 80% imidazole was applied to the clipped dorsal skin (ca. 2x2 cm) and covered by an occlusive dressing. Exposure time was 1 hr in the first experiment using 4 animals (1 male, 3 females); and 4 hr in a second experiment with 2 females. After removal of the occlusion excess material was removed and the skin was cleaned.
EXAMINATIONS
Animals were inspected for skin reactions and signs of general toxicity. The application site was inspected for local signs of inflammation at 1 hr and daily on working days thereafter. Skin reactions, i.e. erythema and oedema were scored similar to the method of Draize:

Score	Erythema	Oedema
0	not present	not present
1	very slight	very slight
2	mild	mild
3	moderate	moderate
4	severe	severe

Test substance: Imidazole, substance no. 77/447; degree of purity approx. 99%

Conclusion: After 4-hr occlusive exposure moderate erythema and oedema were noted within 1 hr. Severe erythema developed overnight and persisted until day 8, the end of the observation period.
Soft necroses developed overnight. They hardened and changed into persistent leather-like necroses in both animals as noted on day 8. Slight oedema were noted at the end of the observation period.
No signs of general toxicity were noted.
Imidazole was corrosive.

Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions (occlusive dressing). Occlusive dressing represents worst case conditions.

Flag: Critical study for SIDS endpoint

17-JUL-2003 (83)

Species: rabbit
Concentration: 80 % active substance
Exposure: Occlusive
Exposure Time: 24 hour(s)
No. of Animals: 3
Vehicle: water
PDII: 5.6

Result: corrosive
EC classificat.: corrosive (causes burns)

Method: Draize Test
GLP: no
Test substance: other TS

Method: Draize test. Skin irritation according to Federal Register 38 no. 178 § 1500.41 p. 27019 (1973) using both intact and scarified skin.
Remark: The Draize test cannot be regarded as a valid test procedure from today's standpoint. It is evaluating scarified skin too, and its exposure time exaggerates the OECD exposure time 6-fold (24 hr) using occlusive dressing instead of semiocclusive dressing. Other than in current test guidelines no readings were made at 1 and 48 hr after treatment.

However, reactions of the intact skin was similar to those of intact skin after 4-h exposure period (BASF 1979a).
Result: Erythema: Severe erythema was noted in all (3/3) animals at the intact (mean score: 3.3) and at the scarified skin (mean score: 4.0) after 24 and after 72 hours. Severe erythema persisted until day 8, the end of the observation period. Necroses were seen as soon as 24h. After 72 h necroses were present at all application sites and persisted until day 8. Necroses were soft at early
stages and hardened during the 8-d observation period to yield leather-like necroses which were confirmed by pathology.
Oedema: Grade 2 oedemas were seen at all application sites after 1, 3, and 8 days.
No signs of general toxicity were noted.
Test condition: TEST ORGANISMS
3 White Vienna rabbits (2 males, 1 female), mean bodyweight ca. 3.0 kg, were used.

ADMINISTRATION
0.5 ml of a water based paste containing 80% imidazole was applied to the clipped dorsal skin (ca. 2.5x2.5 cm) and covered by an occlusive dressing. The skin was intact on one side and scarified at the other site of application. Exposure time was 24 hr. After removal of the occlusion excess material was removed and the skin was cleaned.
EXAMINATIONS
Animals were inspected for skin reactions and signs of general toxicity. The application site was inspected for local signs of inflammation at 1, 3, and 8 days. Skin reactions, i.e. erythema and oedema were scored similar to the method of Draize:

Score	Erythema	Oedema
0	not present	not present
1	very slight	very slight
2	mild	mild
3	moderate	moderate
4	severe	severe

A primary dermal irritation index was calculated as follows: For intact and scarified skin the mean scores for erythema and oedema from all three animals was calculated at both 24

and 72 hr. Thus 8 mean values were obtained. The sum of these 8 means divided by 4 equals the primary irritation index.

Test substance: Imidazole, substance no. 77/447; degree of purity approx. 99%

Conclusion: Invalid study due to overcome methodology. However, results are very similar to those described in the critical Robust Study Summary for this endpoint.

Reliability: (3) invalid
Unsuitable test system with respect to prolonged exposure time, abraded skin, and occlusive dressing.

17-JUL-2003 (84)

Species: rabbit
Concentration: 80 % active substance
Exposure: no data
Exposure Time: 60 minute(s)
No. of Animals: 12
Vehicle: water
Result: corrosive

Method: other: BASF-Test
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Valuable early study, despite limitations exist compared to OECD 404 (no amount of TS given; occlusive exposure; exposure times;
no examination time intervals given). This study is outdated by a new guideline study from the same laboratory with comparable results and detailed study information.

Result: Following a 5 min exposure period there were no findings in any case.
After 15 exposure slight erythema was noted when 50% imidazole was applied. No skin reactions were seen at 80% of the technical grade TS, whereas moderate erythema and eschar formation was noted with 80% of pure TS.
After 60 min of exposure to 50 imidazole moderate erythema were noted which subsided within few hours. 80% imidazole caused severe erythema and oedema; the pure TS additionally caused necroses, desquamation and eschar formation.

Test condition: TEST ORGANISMS
Total number of White rabbits used during skin and eye irritation testing was 12.

ADMINISTRATION
Unknown quantities of water based paste containing imidazole were applied to the clipped intact dorsal skin. Both technical grade and pure imidazole were tested at a total of 52 application sites. Concentrations of 50% and 80% were used. At 50%, pH was adjusted to pH 10 and pH 7. Exposure times were 5, 15, and 60 min. Excess material was removed and the skin was cleaned.
EXAMINATIONS

Skin reactions, i.e. erythema and oedema were examined. No information is provided regarding time intervals and repetitions.

Test substance: Imidazole, 95% technical grade and 100% pure

Conclusion: Contact of 50% imidazole suspension to skin over periods of 5 to 60 min caused transient moderate erythema. 80%

imidazole suspension caused severe erythema, oedema, eschar formation and necroses after 60 min contact to skin.

Reactions to pure imidazole were more pronounced compared to technical TS as evidenced by the earlier appearance of eschar (after 15 min) and necroses after 60 min exposure.

Reliability: (2) valid with restrictions
Limitations exist compared to OECD 404 (no amount of TS given; application mode unknown; no examination time intervals given).

Flag: Critical study for SIDS endpoint

17-JUL-2003 (78)

5.2.2 Eye Irritation

Species: rabbit
Concentration: .1 other: ml
Exposure Time: unspecified
Comment: not rinsed
No. of Animals: 3
Vehicle: none
Result: irritating
EC classificat.: risk of serious damage to eyes

Method: Draize Test
GLP: no
Test substance: other TS

Method: Draize test; eye irritation according to Federal Register 38 no. 178 § 1500.42 p. 27019 (1973).

Result: Cornea: Slight opacity (grade 2) was noted throughout the observation period of 8 d, i.e. this effect persisted. The affected area was more than 3/4, i.e. grade 4. The resulting score was 40 (theoretical maximum attainable: 80) on all examinations.
Iris: A slight inflammation persisted during days 1 to 8. Score was 5 (theoretical maximum attainable: 10).
Conjunctiva: Reddening (grade 2), swelling (grade 2-3) and chemosis (grade 1 after 24 h, grade 3 days 2 through 8) were noted. Scores ranged 10 to 14.
Overall, mean score of all three animals was 57.4 (theoretical maximum attainable: 110).
Other changes noted:
Irreversible changes included greyish/white colouring of the nictating membrane in all animals, scaring of the lid and eye lid hair loss in most animals.

Test condition: TEST ORGANISMS
White Wiener rabbits (2 male, 1 female) were used. Mean body weight was 2.8 kg.
ADMINISTRATION
0.1 ml of Imidazole was placed into the right eye.
EXAMINATIONS
Reactions were examined after 24, 48 and 72 h. Examinations were continued until day 8 if there were persistent reactions.
EVALUATION
According to the regulation a score table was used to evaluate reactions of cornea, iris, and conjunctivae. Theoretical total maximum score was 110.

Test substance: Imidazole, purity approx. 99%
Conclusion: According to the rating scheme the TS was classified as "irritating". This attributes to a mean score of >55 on the scale from 0 to 110.

The observed persisting cornea opacity and other noted irreversible changes indicate the potential of severe eye injuries after contact with TS. Degree of injury, the large area affected and the persistence of the injury need to be taken into account for safety measures.
Reliability: (2) valid with restrictions
Restriction: no reading after 1 hour reported
Flag: Critical study for SIDS endpoint
17-JUL-2003 (85)

Species: rabbit
Concentration: 100 mg
Exposure Time: unspecified
Comment: not rinsed
Vehicle: other: none; solutions: water
Result: irritating

Method: other: BASF-Test
GLP: no
Test substance: other TS

Remark: Valuable early study despite limitations (number of animals examined not given). This study was intended to compare unneutralized with neutralized aqueous preparations with respect to differences in irritation potential.
The observed cornea opacity indicated the potential of severe eye injuries after contact with TS which needs to be taken into account for safety measures.
Result: 80% imidazole caused severe reddening and swelling of conjunctivae and a marked diffuse cornea opacity. The effects resolved within 10-12 days. No difference was noted between pure and technical grade TS.

50% solution adjusted to pH 10 essentially caused the same effects, whereas reactions provoked by the 50% solution adjusted to pH 7 were much less irritant and did not cause any cornea opacity.
Test condition: TEST ORGANISMS
White rabbits were used. Number not detailed. Total number of rabbits used during skin and eye irritation testing was 12.
ADMINISTRATION
0.1g of ground pure and technical grade TS was placed into the eye. 0.1 ml of 50% solutions adjusted to pH 7 and pH 10 were instilled into the eye.
EXAMINATIONS
Reactions were examined after 10 min, 1, 3, and 24 h, and daily until reversibility.
Test substance: Imidazole, 95% technical grade and 100% pure
Conclusion: Early study; indicates irritancy of imidazole powder and 50% solutions at elevated pH 10, less irritant at physiologic pH 7.
Reliability: (2) valid with restrictions
As a minor restriction the number of animals used was not reported in detail.
12-JUL-2003 (78)

Species: rabbit
Concentration: 100 %
Dose: 100 other: mg
No. of Animals: 3
Result: irritating

Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Test substance: other TS

Method: Compilation of data from in-vivo guideline studies according to OECD 405 for the purpose of ranking chemicals according to their MMAS (Modified Maximum Average Score) in order to establish a reference data base for the development of alternative in-vitro methods.
Result: MMAS (Modified Maximum Average Score) of imidazole: 59.3

Imidazole was irritant based on cornea opacity grade 3 and grade 2 effects on iris in one animal after 14 days.
Test condition: TEST ANIMALS
3 rabbits
EXPOSURE
100 mg test substance were placed into the conjunctival sac.
OBSERVATIONS
Examinations of the cornea, iris, and conjunctiva were performed after 1 hour, and after 1, 2, 3, 4, 7, and 14 days.
EVALUATION
The grading scale according to Draize was used. MMAS (Modified Maximum Average Score) was calculated from the results at 24 h or thereafter.
Test substance: Imidazole from Aldrich, purity 99%

Reliability: (1) valid without restriction
Review and collection of existing data. Though secondary literature, the reliability is regarded as being maximal (RL=1) because (1) guideline studies according to OECD 405 were assessed and referenced, and (2) test results for imidazole are reported in full detail.
Flag: Critical study for SIDS endpoint
17-JUL-2003 (86) (87)

5.3 Sensitization

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: 28 days
Frequency of treatment: 5/wk
Post exposure period: none
Doses: 0; 62.5; 125; 250; 500 mg/kg bw/d
Control Group: yes, concurrent no treatment
NOAEL: 62.5 mg/kg bw

Method: other
GLP: no

Test substance: other TS

Method: Subacute toxicity, 28 day gavage

Result: Mortality: None at any dose
Clinical signs:
No clinical signs were noted at dose levels of 62.5 und 125 mg/kg body weight and day. Increased salivation was seen in all animals receiving
250 mg/kg/d after day 17. At 500 mg/kg all animals showed unsteady gait, marked salivation (scattered occurence of blood) from day 15 onwards, and unkempt fur from day 17 onwards.

Food intake, body weight development:
No statistical significant changes at any dose level in male animals. Food intake and terminal body weight was statistically significantly increased in females at 125, 250, and 500 mg/kg body weight and day.

Blood parameters, clinical chemistry:
Statistically significant changes were seen for blood parameters and biochemical analyses as follows:
Hemoglobin reduced in females from 125 ($p<0.05$) to 500 mg/kg body weight and day ($p<0.01$), and in high dose males ($p<0.05$). Hematocrit reduced in females at 250 and 500 mg/kg body weight and day ($p<0.05$ both) and in high dose males ($p<0.05$). Reduced red blood cell count in females at 250 and 500 mg/kg body weight and day.
Creatinine and ALT were both increased in high dose male rats.

Urinalysis revealed no treatment-related changes.

Gross pathology:
Hepatomegaly was noted in most male rats from 125 mg/kg body weight and day onwards, and in 5 of 10 high dose females. Similar findings were obtained during examination of kidneys. A faint grading pattern was noted in kidneys from males exposed to 125 mg/kg body weight and day. This phenomenon was more pronounced at higher doses, but absent in female rats.

Pathology:
Relative organ weight increases (statistically significant) were noted in liver (male rats; 1.9, 11.7, and 14.8% at 125, 250, and 500 mg/kg body weight and day, resp. ($p<0.01$); female rats; 19.1, 14.9, and 33% at 125, 250, and 500 mg/kg body weight and day, resp. ($p<0.01$)); kidney (male rats, 7.1 and 9.5% at 250 and 500 mg/kg body weight and day, resp.), adrenals (high dose male rats), and heart (high dose female rats).

Histopathology:
No changes were noted in lung, liver, heart, kidney, testes, and ovaries that could be related to treatment.

Test condition: TEST ANIMAL
10 animals per sex and dose were used. The animals were 42 days old when the treatment was started, mean body weights were 136 and 127 g for male and female rats, respectively.
TREATMENT
The animals received TS dissolved in water, or vehicle only,

5 days/week by gavage at a volume dose of 10 ml/kg body weight.
EXAMINATIONS and OBSERVATIONS
Clinical signs:
Daily observations for clinical signs and mortalities.
Food intake, body weights:
Food intake was recorded daily, body weights twice per week.

Clinical pathology:
5 animals per test group and sex were used for examination of parameters of hematology (hemoglobin, red and white blood cells, differential blood examination), clinical pathology (sodium, potassium, calcium, chloride, phosphate, carbon dioxide, glucose, urea, protein, lipids, bilirubin, creatinin, alcaline phosphatase, and alanine aminotransferase, ALT), and urinalysis (pH, sediment. glucose, protein, urobilinogen).
Pathology:
After terminal sacrifice the animals were subjected to gross pathology. Heart, liver, kidney, spleen, thyroid, adrenals, and testes were weighed and relative weights were calculated. Samples from multiple tissues were fixed (heart, liver, spleen, kidney, testes/ovaries, thyroid, adrenals, pituitary, brain, lung, pancreas, stomach, small and large intestine, lymph nodes, urinary bladder, skin, tongue, skull, salivary gland, musculus masseter, thymus, trachea, oesophagus, aorta thorica, prostate/uterus, sperm vesicle, epididymes, fatty tissue.
Routine histological examination of heart, lung, liver, and kidney was performed.
STATISTICAL EVALUATION
Means and standard deviation was calculated. Analysis of variance was performed, and significant treatment related effects (s>95%) were subjected to the t-test.

Test substance: Imidazole, purity approx. 99%
Reliability: (2) valid with restrictions
Pre-guideline study with limited examination.
Flag: Critical study for SIDS endpoint
04-APR-2004 (88)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: Wistar
Route of administration: gavage
Exposure period: 90 d
Frequency of treatment: 7/wk
Post exposure period: none
Doses: 0, 20, 60, 180 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: = 60 mg/kg bw

Method: OECD Guide-line 408 "Subchronic Oral Toxicity - Rodent: 90-day Study"
Year: 1998
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: OECD 408 including functional observations, motor activity, and sperm parameters for male reproductive organ function
Remark: (1) Effects on red blood cells similar to those seen in the early 4-week study (BASF 1976) were seen in preliminary

range-finding investigations [not reported] of the 90 day study when a 4-week exposure of Wistar rats showed similar results at comparable (high) dose levels of 250 mg/kg bw and above. The marginal effects in the old 4-week study at 125 mg/kg bw should not be regarded as substance related, as they were not confirmed even after 90-day exposure in this new 90-day study (BASF AG 2002) at higher dose levels (180 mg/kg bw per day).

Result:

Chemical analyses
Stability of TS solutions over a period of 8 days was confirmed. Concentration control analyses yielded 92-105% of the nominal values.

1. Clinical observations
Mortality: one control male died on day 40; one low dose male was sacrificed moribund on d 86.
Clinical examinations: no finding observed that could be related to treatment.
Food and water intake: unaffected except from a transient statistical significantly reduced (-8%) food intake in low dose females on day 14. Not regarded as being substance-related.
Body weights: few statistical significant, but spontaneous body weight increases were noted in both sexes at various times during treatment. Regarded as spontaneous and not substance-related. The same applies to food efficiency.
Ophthalmological examinations: no substance-related effects noted.
Functional observation battery, FOB: Deviations from "zero" was noted in all animal groups including control regarding home cage and open field observations, sensimotor tests and reflexes, and quantitative parameters pertaining to grip strength, landing foot-splay etc.. No dose-relation was noted and observations were equally distributed between control and treated groups. Therefore, no substance-related effect was observed.
Motor activity: no substance-related effect noted.
Estrous cycle determination: no substance-related effect noted.

2. Clinical pathology
Hematology revealed no substance-related effects.
Clinical chemistry: no treatment-related changes except decreased chloride and serum globulins in animals of both sexes at 180 mg/kg/d, and decreased protein and albumin values in high dose females.
Urinalysis: at the end of the study urine sediments of high dose animals revealed increased numbers of transitional epithelial cells in both sexes. Most of these cells were degenerated. No other treatment-related changes were noted.
Sperm analysis: no treatment related changes noted at any dose level.

3. Pathology
Significant effects on absolute or relative organ weight changes were only noted in high dose animals at 180 mg/kg/d. Target organs were liver (relative weights +7.5% in males, +2.6% in females) and kidney (relative weight + 9.1% in males)
Only few scattered gross lesions were noted. No relation to

treatment was seen.

Histopathology
Treatment related microscopic findings were noted in kidneys and liver. They consisted of a slight or moderate diffuse accumulation of a2µ-microglobulin (1/1/1/10) in the epithelia and lumina of the proximal tubules of the renal cortex, as shown by the widely used Mallory-Heidenhain staining. Specificity for a2µ-microglobulin could be demostrated by immunohistochemical staining. The microglobulin accumulation was not associated with any further alterations of the tubular epithelium. It was regarded as a substance-related effect in high dose males.
Minimal or slight centrolobular hypertrophy of liver cells was noted in males (0/0/0/9) and in females (0/0/0/2) which correlated with increased absolute (females) and relative (males and females) liver weights. No other histological or morphological effects in the liver were noted.

Test condition: TEST ORGANISM
A total of 100 animals, 10 rats per dose/sex were used, age 42+/-1 d at start of administration period. Body weight ca. 150g for male and 120g for female rats. Animals were housed individually.
OVERALL EXPERIMENTAL DESIGN
Animals were given imidazole daily by gavage for 3 months and killed thereafter. Clinical examinations, clinical chemistry, gross pathological and histopathological changes were examined in various organs including testes as prescribed by OECD 408. Ophthalmological and behavioral (functional observation battery, motor activity) examinations were performed. Additionally, sperm parameters were examined.
ADMINISTRATION
Dose levels were selected based on the results of a 4-week range finding study (data filed under 31S0694/00094 but not reported). Imidazole was dissolved in bidistilled water. Dose volume was 10 ml/kg bw.

EXAMINATIONS/OBSERVATIONS
1. Clinical observations
Food and water intake and body weight were determined weekly. Body weight was determined prior to randomization at the start of the administration period and weekly thereafter. Body weight gain and food efficiency (body weight gain relative to food intake) were calculated from the recorded data.
Ophthalmoscopical examination of all animals was performed prior to the treatment period, and of control and high dose animals on day 91 using an ophthalmoscope and a mydriatic.
Functional observation battery (FOB) included home cage and open field observations along with sensimotor and reflex tests. Motor activity was assessed using an automated infrared beam system.
Estrous cycle determinations by examination of vaginal smears were conducted during study days 63 and 91.
2. Clinical pathology
a) Hematology (leukocytes, erythrocytes, hemoglobin, hematocrit, means of corpuscular volume, hemoglobin, and corpuscular hemoglobin, platelets, and differential blood count; clotting analysis); clinical chemistry (alanine and

aspartate aminotransferases, alkaline phosphatase, serum g-GT, sodium, calcium, magnesium, potassium, chloride, inorganic phosphate, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, and cholesterol. b) Urinalysis comprised examination of volume, color, turbidity, pH, specific gravity, sediment, protein, glucose, ketones, urobilinogen, bilirubin, and blood.
c) Sperm parameters were determined in all males at termination. Right testis and cauda epididymis were taken and weighed. Sperm count in testis and epididymis, motility and sperm morphology were determined.
3. Pathology
Animals were sacrificed on days 92 and 93 and assessed by gross pathology. Organ weights were determined of organs as required by OECD 408 including reproductive organs, and additionally of thymus and prostate gland.
Tissues were fixed in 4% formaldehyde for histopathology: all gross lesions, salivary glands, esophagus, stomach, small and large intestine, liver, pancreas, brain, pituitary gland, sciatic nerve, spinal cord, eyes, adrenals, thyroid glands, parathyroid glands, trachea, lungs, pharynx, larynx, nasal cavities, aorta, heart, bone marrow, lymph nodes, spleen, thymus, kidneys, urinary bladder, ovaries, oviducts, uterus, vagina, prostate gland seminal vesicles, female mammary gland, skin, skeletal muscle, sternum with marrow, femur with knee joint, lacrimal glands. All tissues were examined in control and high dose animals. Gross lesions and kidneys were examined in all animal groups.
Mallory-Heidenhain staining was used to detect a2u-microglobulin. Specificity of the Mallory-Heidenhain staining for a2µ-microglobulin was examined using a non-commercial antibody (donation of Bayer AG). The kidneys from 3 male controls and 3 male high dose animals were sectioned, histochemically stained and assessed by light microscopy. Untreated sections served as negative control (Amendment No. 1 to the report; BASF 2004).

STATISTICAL EVALUATION
Evaluation of clinical data (body weight, bw changes, food and water intake, food efficiency) was done using DUNNETT's test. Non-parametric one-way analysis using KRUSKALL-WALLIS test was used to evaluate FOB and motor activity data. If p-values were equal or less than 0.05, WILCOXON-test was also used.
Non-parametric one-way analysis using KRUSKALL-WALLIS test was also used to evaluate clinical pathology parameters.

FISHER'S exact test was included to evaluate some of the urinalysis parameters. WILCOXON-test was used for evaluation of sperm parameters.
Non-parametric one-way KRUSKALL-WALLIS test and WILCOXON-test was also used to evaluate absolute and relative organ weights.

Test substance: Imidazole, degree of purity 99.8 %

Conclusion: Imidazole was tested in a thorough 90-d study according to OECD 408 including several functional tests (FOB, motor activity, ophthalmological examinations, sperm parameters).

Liver and kidney were identified as target organs. At the high dose (180 mg/kg/d) significant and substance-related

changes noted consisted of centrolobular liver cell hypertrophy in both sexes (9/10 males and 2/10 females); diffuse a2u-microglobulin accumulation in proximal tubules of the renal cortex in male rats; increased absolute (females) and relative (males and females) mean liver weight; increased absolute and relative mean kidney weight in male rats; and blood chemistry changes (decreases in serum protein and albumin in females, and in chloride and globulins in both sexes).

No other substance-related finding was noted. This includes the histopathological examination of reproductive organs of both sexes, and sperm parameters examined as an indicator of the integrity the male reproductive organs.

Since the substance-related findings described above were limited to the high dose group animals and absent in low dose and intermediate dose group animals, the no observed adverse effect level (NOAEL) for both sexes was 60 mg/kg/d in this study.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
19-MAR-2004 (89) (90)

5.5 Genetic Toxicity 'in Vitro'

Type: Bacterial gene mutation assay
System of testing: Klebsiella Pneumoniae
Metabolic activation: without
Result: negative

Test substance: other TS

Remark: A second reference of the same author was originally contained in the Iuclid data set. This reference was taken out because the subject of this publication was the mutagenic action of 50 substituted imidazoles. Imidazole itself was also included, but no results were reported. This study is therefore invalid for the assessment of imidazole (Voogd CE, van der Stel JJ, Jacobs JJJAA (1979) The mutagenic action of nitroimidazoles. IV. A comparison of the mutagenic action of several nitroimidazoles and some imidazoles. Mutat Res 66: 207-221).
Test substance: Imidazole (unspecified purity) along with several methyl- and nitroimidazoles
Reliability: (4) not assignable
Abstract
13-JUL-2003 (91)

Type: Ames test
System of testing: Salmonella typhimurium TA1535, TA1537, TA1538, TA100, TA98
Metabolic activation: with and without
Result: negative

Method: other
GLP: no data
Test substance: other TS

Reliability: (4) not assignable

Scientific publication without detailed documentation.
Secondary literature.
12-JAN-2004 (92)

Type: Bacterial gene mutation assay
System of testing: Escherichia coli WP2 Hcr-
Metabolic activation: without
Result: negative

Reliability: (4) not assignable
Scientific publication without detailed documentation.
Secondary literature.
12-JAN-2004 (92)

Type: Bacterial gene mutation assay
System of testing: Bacillus subtilis R17(Rec+); M45(Rec-)
Metabolic activation: without
Result: negative

Reliability: (4) not assignable
Scientific publication without detailed documentation.
Secondary literature.
12-JAN-2004 (92)

Type: Ames test
System of testing: Salmonella typhimurium TA1535, TA100, TA1537, TA98
Concentration: 0, 20, 100, 500, 2500, 5000 µg/plate
Cytotoxic Concentration: > 5000 µg/plate
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 1983
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Standard plate (accord. to Ames et al.) and preincubation test (accord. to Matsushima et al.)
Result: Complete solubility of TS in aqua dest. was given.
No bacteriotoxic effect (reduced his- background growth) was noted.

No increase of revertants was seen in the standard plate or preincubation test, with or without S9 mix, in any tester strain.
The mean revertant increase by positive control substances was given.
Test condition: TEST SYSTEM
Base pair substitution (TA 1535, TA 100) and frameshift (TA1537, TA 98) tester strains with and without metabolic activation. Standard plate test (according to Ames et al.) and preincubation test (according to Matsushima et al.) were performed.
In the standard plate test, 0.1 ml of bacteria suspension (1E+06 bacteria/ml) is given to 2 ml of soft agar containing 0.5 mM histidine; 0.1 ml of test solution and 0.5 ml of S9 mix (or phosphate buffer) is added, mixed and poured onto minimal glucose agar plates. After incubation at 37°C for 48 hrs in the dark, the number of his+-revertants is counted.
The preincubation test differs in that bacteria are preincubated with S9 mix and the test substance solution at

37°C for 20 min prior to mixing with soft agar and incubation on the agar plates.
ADMINISTRATION
Doses:
1st experiment: standard plate test
0, 20, 100, 500, 2500, 5000 µg/plate tested with all tester strains. Solvent was aqua dest. 3 plates per dose and control.
2nd experiment: preincubation test
0, 100, 500,2500, 5000, 7500 µg/plate tested with TA 100. 3 plates per dose and control.
Positive controls:
2.5 and 10 µg 2-aminoanthracene (+S9, all strains); -S9: 5 µg MNNG (TA 100, TA 1535), 10 µg 4-nitrophenylenediamine (TA 98), 100 µg 9-aminoacridine chloride monohydrate (TA 1537). DMSO was used as solvent.

EVALUATION CRITERIA
Reaction to a substance is regarded as positive if all of the following criteria are met
- doubling of spontaneous control mutation rate
- dose-response relationship
- reproducibility of results

Test substance: Imidazole, purity 99.9%
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
21-OCT-2003 (93)

Type: Ames test
System of testing: S. typhimurium tester strains TA97, TA98, TA100, TA102
Concentration: 0.625, 1.25, 2.5, 5, 10 mg/plate
Cytotoxic Concentration: > 10 mg/plate
Metabolic activation: with and without
Result: negative

Method: other: equivalent to OECD guideline 471
Test substance: other TS

Remark: The metabolites hydantoin, hydantoic acid, and N-acetyl-imidazole were equally negative and showed no cytotoxicity when tested in the Ames test at dose levels of 0.1 to 10 mg/plate.
Result: Compared to the negative control the average number of revertants (mean of two independent experiments) was not increased by imidazole neither with nor without metabolic activation at concentrations up to and including 10 mg/plate, whereas the positive control substances led to increased numbers of revertants as expected:

Dose level mg/plate	TA97	TA98	TA100	TA102
Without S9:				
0	131	35	164	351
0.625	143	35	159	355
2.5	147	31	167	353
10	142	37	166	346
pos. ctrl.	422	292	726	831
With S9:				

0	158	51	184	420
0.625	161	49	179	441
2.5	162	43	177	450
10	127	45	199	428
pos. ctrl.	1950	1245	1531	1816

(data for dose levels 1.25 and 5 mg/plate omitted for clarity)

No cytotoxicity was noted even at the highest dose tested.

Test condition: TEST SYSTEM
Standard plate test with and without metabolic activation by rat liver S9 homogenate from rats pretreated with ß-naphthoflavone and phenobarbital.
Negative controls (distilled water) and positive controls were included.
ADMINISTRATION
625 to 10,000 µg/plate were added. Two independent experiments with 3 plates per test point were performed.
EVALUATION
Average number of revertants (mean of two independent experiments) is reported.

Test substance: Imidazol >99% from Fluka, and hydantoin, hydantoic acid, and N-acetyl-imidazole

Conclusion: Imidazole and its metabolites showed no mutagenicity in bacterial in-vitro test systems in the presence or absence of metabolic activation.

Reliability: (1) valid without restriction

Flag: Critical study for SIDS endpoint

17-JUL-2003 (94)

Type: Unscheduled DNA synthesis
System of testing: Rat primary hepatocytes
Concentration: 0.25, 0.5, 1, 2, 4 mg/ml
Cytotoxic Concentration: approx. 1 mg/ml for 50% cell survival
Metabolic activation: without
Result: negative

Method: other: equivalent to OECD Guide-line 482

Method: Incorporation of tritiated thymidine into DNA with subsequent audioradiography and microscopic silver grain count.

Result: Results of 3 independent experiments were similar to those below (from the 2nd experiment)

Treatment	Net Nuclear Grains	(%positive)	% Survival
Control	-2.1	(5)	100
Imidazole			
0.25 mg/ml	0.2	(20)	100
0.5 mg/ml	-1.0	(30)	79
1.0 mg/ml	0.4	(15)	48
2.0 mg/ml	2.1	(35)	25
4.0 mg/ml	too toxic		2
DMBA	25.0	(100)	52
UV light	38.8	(100)	32

All over all, imidazole did not induce UDS in primary rat heaptocytes. Cytotoxicity differed in the 3 experiments. 50% survival was noted at approx. 1 mg/ml.

Test condition: The test was based on the method described by Willims , Laspia, and Dunkel (1982) Mut Res 97:359-370
TEST SYSTEM
Primary hepatocytes from untreated male Wistar rats were used. A single animal was used for each experiment. Tests were performed in duplicate. 3 independent experiments were performed.
ADMINISTRATION/DOSE LEVELS
TS was added to the incubation medium during the 18-hr incubation period at the indicated dose levels. Negative and two positive controls (7,12-Dimethylbenzantracene and UV-light) were included.
EVALUATION
Cover slips were washed, fixed on slides and dried after the incubation period. After autoradiograpy and cells were stained with hematoxylin and eosin the number of nuclear and cytosolic grains was evaluated for approx. 20 cells/test point under the microscope at 1000-fold magnification. The test treatment was considered positive if a dose-related increase and values of greater than +3.0 silver grains were achieved.
A third dish was used to evaluate cytotoxicity which was estimated using Trypan blue exclusion.

Test substance: Imidazole >99% from Fluka
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
17-JUL-2003 (94)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: NMRI
Route of admin.: gavage
Exposure period: single dose
Doses: 500, 1000, 2000 mg/kg bw (suspended in 10 ml olive oil/kg bw)
Result: negative

Method: OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year: 1983
GLP: yes
Test substance: other TS

Method: Genetic toxicity in vio. In accordance with OECD test guideline 474 for Micronucleus test, and EEC Directive 84/449 B12, Micronucleus Test

Result: Range finding study:
During pretests deaths were observed down to a dose of 2250 mg/kg bw whereas 2000 mg/kg bw was survived by all animals but led to signs of toxicity including irregular respiration, piloerection, squatting posture in some cases, closed eyelids, and saltatory convulsions.

Main study:
Analytical examination of olive oil samples revealed concentrations of 52.9, 100.4, and 198.0 g/l, i.e. 99-106% of the theoretical values. Substance stability was verified.

Clinical signs:
none after vehicle or after positive control substances. Signs following TS at 500 mg/kg bw: irregular respiration and piloerection 15-30 min after dosing; at 1000 mg/kg bw additionally closed eyelids in some cases within 15-30 min; at 2000 mg/kg bw: additionally squatting posture in some animals; one high dose animals was found dead within 1 h after dosing. No signs were noted in positive control animals.

Mean micronuclei counts at 24 hr after dosing, expressed as 0/oo in polychromatic (=pe) and total number of normochromatic (=ne) erythrocytes containing micronuclei:

Solvent control:	pe= 2.6	ne= 0.3
cyclophosphamide:	pe= 8.6	ne= 1.6
vincristine:	pe= 15.4	ne= 3.0
TS, 500 mg/kg bw:	pe= 2.2	ne= 0.4
TS, 1000 mg/kg bw:	pe= 1.7	ne= 0.4
TS, 2000 mg/kg bw:	pe= 2.3	ne= 0.3
Other time intervals		
TS, 2000 mg/kg bw, 16 h;	pe= 1.9	ne= 1.1
TS, 2000 mg/kg bw, 48 h;	pe= 1.4	ne= 0.9

Compared to control animals imidazole did not change the incidence of micronuclei in polychromatic or normochromatic erythrocytes at any time or dose. The ratio of polycromatic to normochromatic erythrocytes was unchanged.

Test condition: TEST ORGANISM
70 NMRI mice, male and female, mean bw 28 g. 5 animals per dose and sex were used for TS and negative control, and 5 animals (2 and 3 per sex) for each of the positive control substances.
ADMINISTRATION
Imidazole was singly administered by oral gavage in olive oil at a dose volume of 10 ml/kg bw. Concentrations were 5, 10,
and 20 g/100 ml oil, resulting in doses of 500, 1000, and 2000 mg imidazole/kg bw. Negative controls received vehicle only. Positive controls received the clastogen cyclophosphamide (20 mg/kg bw, in water, gavage at 10 ml/kg bw) or
the spindle poison vincristine (0.15 mg/kg bw, in water, i.p.
at 10 ml/kg bw).
EXAMINATION

The animals were examined for clinical signs of toxicity after dosing. Femural bone marrow was prepared from all control and dose group animals at 24 hr post dosing, and additionally at 16 hr and 48 hr in animals receiving the high 2000 mg/kg bw dose.
EVALUATION CRITERIA
Generally, 1000 polychromatic erythrocytes per animal are evaluated for the parameters: (1) no. of polychromatic erythrocytes with/without micronuclei and calculation of clastogenic index, (2) no. of normochromatic erythrocytes with/without micronuclei, calculation of ratio polychromatic/normochromatic erythrocytes, (3) no. of small(d<1/4) and large (d>1/4) micronuclei.

No statistical data analysis was necessary to be performed. The no. of polychromatic micronucleated erythrocytes after TS treatment was nearly the range of the actual control value, and within the historical values.

Test substance: Imidazole as hydrochloride, degree of purity 99.5 %

Conclusion: No significant or biologically relevant differences were noted between treated and solvent control group animals.

Thus, imidazole showed no clastogenic (DNA-damaging) property, nor did it impair chromosome distribution due to a spindle poison effect.

Reliability: (1) valid without restriction

Flag: Critical study for SIDS endpoint

17-JUL-2003 (95)

5.7 Carcinogenicity

16-DEC-2003

5.8.1 Toxicity to Fertility

Type: other: reproductive organs, sperm parameters
Species: rat
Sex: male/female
Strain: Wistar
Route of administration: gavage
Exposure Period: 90 d
Frequency of treatment: 7/d
Duration of test: 3 mon
Doses: 0, 20, 60, 180 mg/kg/d
Control Group: yes, concurrent vehicle
NOAEL Parental: = 60 mg/kg bw
other: male and female reproductive organs : = 180 mg/kg bw
Result: male reproductive organs and sperm parameters not changed at 180 mg/kg/d

Method: other: OECD 408
Year: 1998
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: OECD 408 including functional observations, motoractivity, and sperm parameters for male reproductive organ function

Result: Sperm parameters were unchanged at any dose level.

During pathological and histopathological examinations no substance-induced changes were noted in any of the examined male and female reproductive organs.

Test condition: Reference is made to the full description of Test Conditions which is provided in the Study Summary in Chapter 5.4, Repeated Dose Toxicity.

In brief, sperm parameters were determined in all males at termination. Right testis and cauda epididymis were taken and weighed. Sperm count in testis and epididymis, motility and sperm morphology were determined.

Histopathological examination of control and high dose males and females included male and female reproductive organs, i.e. left testis, left epididymis, prostate gland and seminal vesicles; ovaries, mammary gland, uterus.

Test substance: Imidazole, degree of purity 99.8 %

Conclusion: The results indicate that imidazole did not influence sperm production and sperm quality parameters in male rats, and that no signs of toxicity were noted in male or female reproductive organs. Thus, no reproductive organ toxicity or hormone-mimetic action of imidazole or one of its metabolites was noted.

The no adverse effect level (NOAEL) of 60 mg/kg/d was based on effects on liver and kidneys observed at 180 mg/kg/d.

With regard to fertility and reproduction, the NOAEL was 180 mg/kg/d based on no observed effects in male and female reproductive organs, and on the unchanged sperm parameters.

Reliability: (1) valid without restriction

Flag: Critical study for SIDS endpoint

17-JUL-2003 (90)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain: Wistar
Route of administration: gavage
Exposure period: 1 day; 12th or 13th day of gestation
Frequency of treatment: single dose
Doses: 240 mg/kg
Control Group: yes

Method: other

Method: Single oral dose on gestational day 12 or 13, followed by sacrifice of dams on day 22 and examination of fetuses.

Remark: Exploratory study. Purpose of the study was to elucidate a correlation of teratogenicity of ethylenethiourea and molecular structure of related compounds, including imidazole.

Result: Imidazole did not cause teratogenicity. No effect on litter size and the number of viable and stillborn pups was noted. The number of pups with visceral and skeletal malformations was not increased.

Reliability: (3) invalid
Single dose administration, single dose level, low number of dams, limited examination of fetuses and dams, limited reporting do not comply with OECD guideline.

17-JUL-2003 (96)

Species: other: rat and mouse embryos **Sex:** no data
Strain: other: Sprague Dawley rats and CD-1 mice
Route of administration: other: in vitro exposure
Exposure period: not indicated, presumably 2 days
Frequency of treatment: continuous
Duration of test: 2 days
Doses: 30 or 60 ug/ml
Control Group: yes, concurrent vehicle

Method: other: whole embryo culture technique

GLP: no data
Test substance: other TS

Remark: (1) Imidazole was included in the test because of its structural relationship to Ethylenethiourea.
(2) Evaluation should be made by using in-vivo testing according to OECD guideline 414.
Result: A concentration-related high mortality up to 83.3 % and a high rate of abnormalities up to 100 % was found in treated embryos.
Treated embryos showed also reduced yolk sac diameter and crown rump length and less somites.
Characteristic abnormalities were decreased brain size and clear blisters. Mouse embryos were generally more sensitive to substance related effects.
Test condition: 6 - 10 embryos were used per species per concentration. The following parameters were examined: mortality, abnormalities, yolk sac diameter, number of somites, crown rump length.
Test substance: Imidazole, no further data.
Reliability: (2) valid with restrictions
In-vitro study (teratogenicity screen).
13-JUL-2003 (97)

Species: rat **Sex:** female
Strain: Wistar
Exposure period: days 6 through 19 post coitum
Frequency of treatment: 7 d/wk
Duration of test: 14 d
Doses: 0, 20, 60, 180 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: = 60 mg/kg bw
NOAEL Teratogenicity: = 60 mg/kg bw
NOAEL Fetotoxicity : = 60 mg/kg bw
NOAEL Embryotoxicity : = 60 mg/kg bw
Result: Prenatal toxicity (embryotoxic, teratogenic) at maternal toxic dose level

Method: OECD Guide-line 414 "Teratogenicity"
Year: 2001
GLP: yes

Result: MATERNAL EFFECTS
Only pregnant dams or pregnant dams sacrificed at schedule were used for calculations. Animals which were totally or partially excluded were: 3 non-pregnant control animals; one non-pregnant animal from each dose group. No mortality was seen in any of the groups.

Low dose, 20 mg/kg bw/d:
No substance related effects seen.

Intermediate dose, 60 mg/kg bw/d:
No substance related effects seen, except slight but significant decrease of body weight gain on d 6-8 p.c.

High dose, 180 mg/kg bw/d:

Clinical data:
Transient salivation was noted in 6/25 animals during days 15-19 p.c. starting few minutes after dosing and lasting 15

to 20 min. Vaginal hemorrhage on d 20 was noted in one animal.
Salivation was regarded to be treatment related due to bad taste or local affection of the upper digestive tract, but was not assessed as an adverse or toxic effect.

Food intake, body weights:
Food intake was significantly reduced (-13% compared to controls) during a period at the beginning of treatment on d 6-8 p.c. Body weight gain was also statistically reduced compared to controls on d 6-8 p.c. (-45%) and d 17-20 p.c. (-34%). However, at the end of the treatment period body weight was comparable in all groups.
Findings were regarded as being substance-induced and reflecting direct adverse effects on dams (d 6-8) and on fetuses (d 17-20; resorptions, lowered fetal body weight).

Corrected body weight gain was comparable in all dose groups.

Terminal examination of dams:
Uterus weight was significantly reduced (-26% vs. controls). Significantly increased postimplantation loss (43% vs 8% in controls) was due to late resorptions. 3/24 females resorbed all implants during the last treatment days and had no live fetuses at termination. The number of live fetuses per litter was significantly reduced. Additionally the number of live male fetuses per litter was significantly reduced.

Examination of fetuses
Sex distribution was comparable to control animals in all treatment groups. In high dose animals, placental weight was increased (+22% vs. control), whereas mean fetal body weight was reduced (-14%, both sexes combined).

External malformations (anasarca and/or cleft palate) were significantly increased in high dose fetuses (13/132 fetuses; ca. 10%) in 7/21 litters (=33%), i.e. 9% versus 0% affected fetuses/litter in the control group.

Soft tissue variations (dilated renal pelvis and ureter) were statistically significantly increased compared with controls (27.1% vs. 6.4%).

Skeletal malformations (shortened scapula, bent radius/ulna, malpositioned and bipartite sternebra) were significantly increased; noted in 7/73 fetuses (=9.6%) in 5/21 litters (=24%), i.e. 7.8% vs. 1.1% affected fetuses/litter in the control group. Also, significant increase of several skeletal variations (mainly delays in the ossification process) was noted (98.4% affected fetuses/litter in high dose group vs. 91.1% in control group).

Test condition: TEST ORGANISM
25 time-mated female rats per dose, age ca. 70 d at beginning of the study (day 0, detection of sperm), were used. Body weights ranged between 143 to 186 g on day o post coitum (p.c.).

ADMINISTRATION
TS was administered by oral gavage once a day from implantation to one day prior to expected parturition, i.e.

d 6-19 p.c. TS dissolved in doubly distilled water was given at 20, 60, and 180 mg/kg bw/d.

EXAMINATIONS

TS: analysis of TS purity and stability was performed; analysis of TS solutions using GC before study started and after it ended.

Dams: During the conduct of the study the animals were examined at least daily for clinical symptoms. Mortality was checked twice a day or once on Saturday/Sunday. Food and water consumption, body weight on days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20 p.c.. Corrected body weight gain was calculated after terminal sacrifice as [bw d 20- (uterus weight+bw d 6 p.c.)].

Examinations at termination:

Dams: Sacrificed on day 20 pc. Necropsy included gross pathology assessment. Uterus and ovaries were removed with subsequent examination and recording of: unopened uterus weight; no. of corpora lutea; no. and classification of implantation sites as live fetuses or dead implantations (early, late resorptions, dead fetuses). Calculation of conception rate and pre- and postimplantation losses.

Fetuses: were weighed and sexed by anogenital distance observation (which was later confirmed by internal examination of those fetuses which were preserved in BOUIN's solution), and macroscopically examined (viability, condition of placenta, fetal membranes, umbilical cords, placental weight). Approx. 50% of all fetuses were subjected to soft tissue examinations after fixation in BOUIN's solution, the other 50% of fetuses was examined for skeletal changes.

EVALUATION
In order to minimize bias, dams were sacrificed in a randomized order, and all examinations subsequent to weighing the unopened uterus were conducted without knowledge of the treatment group.

The glossary of Wise et al. (1997) and the definitions proposed by Chahoud et al. (1999) were used during assessing fetal findings. Thus, "malformation" was used for a permanent structural change that is likely to adversely affect the survival or the health; "variation" is used for a change that also occurs in fetuses of control animals and is unlikely to adversely affect survival or health. This includes delays in growth or morphogenesis that has otherwise followed a normal pattern of development. "Unclassified observation" or "unclassified cartilage observation" were used if fetal findings could not be classified as malformations or variations.

Statistical evaluation of data included FISHER's Exact Test for conception rate, mortality of the dams, and number of litters with fetal findings; WILCOXON Test for proportions of fetuses with malformations and/or variations in each litter; and DUNNETT's Test for all other data including

water consumption and body weight gain.

Test substance: Imidazole, degree of purity 99.8 %
Conclusion: Maternal toxicity was noted exclusively at 180 mg/kg bw/d as substantiated by significantly reduced food intake at initiation of treatment and impaired body weight gains on days 6-8 p.c.. No signs of maternal toxicity were seen at 60

mg/kg bw/d and below.

Fetal development was adversely affected at 180 mg/kg bw/d as
substantiated by the high rate of late resorptions (3/24 animals showing complete resorption) which resulted in an elevated postimplantation loss and reduced fetal body weights.

At 180 mg/kg bw/d selective teratogenicity was indicated by increased occurrence of external and skeletal malformations and variations of which anasarca, cleft palate, shortened scapula, and incomplete or delayed ossifications were the most prominent. No increases were noted at 20 or 60 mg/kg bw/d.

Based on these findings NOAEL for maternal toxicity and for prenatal developmental toxicity is 60 mg/kg/d
Reliability: (1) valid without restriction
1a-GLP guideline study
Flag: Critical study for SIDS endpoint
06-APR-2004 (98)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

Endpoint: Neurotoxicity
Study described in chapter: 5.4 Repeated Dose Toxicity
Reference: BASF AG, Department of Toxicology, report no. 51S0694/00123, 15 Sep 2002
Type: other: 90 d-study
Species: rat
Strain: Wistar **Sex:** male/female
Route of administration: gavage
No. of animals: 100
Vehicle: water
Exposure Period: 90 day(s)
Frequency of treatment: 7/wk
Doses: 0, 20, 60, 180 mg/kg bw/d
Control Group: yes, concurrent vehicle
Observation Period: none
Result: negative

Method: other: OECD 408
Year: 1998
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Reliability: 1a - GLP guideline study
Result: No behavioral changes were noted at any of the dose levels

including 180 mg/kg bw/d during the numerous tests performed
in the functional observation battery, FOB, and the motor activity.

Test condition: Reference is made to the full description of Test Conditions which is provided in the Study Summary in Chapter 5.4, Repeated Dose Toxicity.

FOB, functional observational batteries, were carried out with all animals at the end of the administration period. The FOB consisted of 4 parts, starting with cage observations (posture, tremor, convulsions, abnormal movements, impairment of gait, etc.), open field observations (fur, skin, behavior, salivation, lacrimation, eyes/pupil size, respiration, posture, tremors, activity, feces, urine etc), and subsequent sensorimotor and reflex tests (e.g. approach response, vision, audition, coordination of movements, pain perception, grip strength, landing foot-splay test, and others).
Motor activity was assessed in the same animals on the same day in the dark using an automated infrared-beam system that measures animals movements during 12 intervals of 5 min each.

Test substance: Imidazole, degree of purity 99.8 %

Conclusion: Continuous administration of imidazole at 180 mg/kg bw/d over a
period of 3 months did not change behavior, motor activity, and sensimotor capabilities and reflexes examined in the functional observation battery.

Since no adverse effect was noted at the highest dose administered, the no observable effect level (NOAEL) was 180 mg/kg bw/d for these endpoints in this study.

16-DEC-2003 (90)

5.10 Exposure Experience

Remark: no data available

31-JUL-2002

5.11 Additional Remarks

Type: Behaviour

Remark: Imidazole at 150 mg/kg bw i.p. caused trembling in Wistar rats. The effect was age and sex dependent, males reacting much stronger. No difference was seen between males and ovariectomized females. The effect was small in young animals and most
evident in animals at the age of 11 to 18 weeks.

13-SEP-2002 (99)

Type: Biochemical or cellular interactions

Remark: New Zealand White rabbits treated with imidazole (200 mg/kg bw, 4 days) showed increased total p450-content in liver (1.24-fold) compared with controls and a 4.47-fold increase of the isozyme 3a.

Reliability: (2) valid with restrictions
Meets accepted scientific standards and is described in sufficient detail
Flag: Critical study for SIDS endpoint

17-JUL-2003 (100) (101) (102)

Type: Biochemical or cellular interactions

Remark: Proliferating B16/C3 melanoma cell cultures were treated with androgens (testosteron, epitestosteron, 5-Hydroxytestosteron), estradiol und estriol.
None of these substances caused changes in enzyme activities.
Imidazole strongly induced tyrosinase, which is involved in melanogenesis in this cell line. The androgens and estrogens mentioned above inhibit this induction.

13-JUL-2003 (103) (104)

Type: Biochemical or cellular interactions

Remark: Inhibition of p-Nitrophenol hydroxylase in rat liver microsomes by small aromatic and heterocyclic molecules

14-MAY-1997 (105)

Type: Biochemical or cellular interactions

Remark: Hepatic microsomes isolated from rats treated with imidazole for 3 days (200 mg/kg bw/day) showed no increase in epoxide hydrolase gene expression.

13-JUL-2003 (106)

Type: Biochemical or cellular interactions

Method: Induction of liver drug metabolism
Remark: Microsomes from rats pretreated with ethanol (liquid diet, 36% of calories) were also examined. Compared with controls, total cytochrome content was increases 2-fold, and all of the four tested enzyme activities were increased 3.2 to 4.88-fold exempt aminopyrine-N-demethylase (1.33-fold).
Result: Compared with microsomes from control animals receiving saline significant ($p<0.05$) increases of 7-ethoxycoumarin-O-deethylase (1.7-fold) and aminopyrine-N-demethylase (1.26-fold) were noted.

No increase in total cytochrome p-450 content was noted. Gel electrophoresis did not indicate any change in P-450 isoenzymes. Activity of aniline hydroxylase and of p-nitrophenol hydroxylase were insignificantly reduced.
Test condition: TEST ANIMALS
3 female Sprague-Dawley rats per group were used.
TREATMENT
Imidazole dissolved in saline was injected i.p. at 200 mg/kg for 4 consecutive days. Thereafter animals were sacrificed and liver microsomes were prepared and tested. Control animals received saline only. Another group was pretreated with ethanol.
EXAMINATIONS

Total cytochrome P-450 content. The content of isozymes was examined using gel electrophoresis and enzyme activity tests.
STATISTICAL DATA TREATMENT
Group means and standard deviation were reported and compared for statistically significant differences (statistical method not reported).

Test substance: Imidazole from Sigma Chemical Co., reagent grade

Conclusion: Pretreatment of rats with imidazole caused slight changes on liver cytochrome P-450 isoenzymes. Effects were different and smaller from those following ethanol pretreatment.

Reliability: (2) valid with restrictions
Exploratory study

Flag: Critical study for SIDS endpoint

19-MAR-2004 (107)

Type: Biochemical or cellular interactions

Method: Induction of liver drug metabolism

Result: No statistically significant changes noted compared to the control animal groups in either sex in any of the parameters exempt a 30% decrease of ethylmorphine demethylation in females.

Test condition: TEST ANIMALS
Male and female Syrian golden hamsters, 3 animals per control and treatment group.

TREATMENT
Imidazole suspended in 30% polyethylene glycol 400 was injected i.p. at 200 mg/kg for 4 consecutive days. Thereafter animals were sacrificed and liver microsomes were prepared and tested. Controls received saline only. Other groups of animals received substituted imidazoles.
EXAMINATIONS
Parameters tested included liver weight, total cytochrome P-450 content, microsomal phase I enzymes (demethylation of p-nitroanisole and ethylmorphine, NADPH-cytochrome C reductase), and cytosolic phase II enzymes (sulfotransferase, glutathione transferase).
STATISTICAL DATA TREATMENT
Group means and standard deviation were reported and compared for statistically significant differences (statistical method not reported).

Test substance: Imidazole from Eastman Kodak, unspecified degree of purity

Conclusion: Treatment of Syrian golden hamsters with imidazole did not influence liver weight and liver phase I and phase II drug metabolism enzymes.

Reliability: (2) valid with restrictions
Exploratory study

Flag: Critical study for SIDS endpoint

19-MAR-2004 (108)

Type: Cytotoxicity

Remark: Reduction of cell protein content was examined in vitro. Imidazole caused a 50% within 24 hr at 45 mM in Hep G2 cells.

13-SEP-2002 (109)

Type: Cytotoxicity

Remark: Cytotoxicity was studied in vitro using rat brain cells (neuronal and non-neuronal cell types were used) taken from fetuses on gestational day 19. Imidazole did not cause degeneration of these cells.

13-SEP-2002 (110)

Type: other: Alternatives to the Draize rabbit eye irritation test (COLIPA study)

Remark: Comparison of 10 alternative methods using 55 test substances
Test substance: Imidazole possibly included
Reliability: (4) not assignable

15-JUL-2003 (111)

Type: other: Bovine cornea opacity and permeability assay

17-JUL-2003 (112)

Type: other: Bovine cornea opacity and permeability assay. Interlaboratory assessment

Method: Interlaboratory comparison of accuracy of an in-vitro test system for eye irritation.
Result: (1) In-vitro test assessment:
Variation between laboratories was low. Reproducibility was not tested.
Comparison with results from in-vivo testing revealed good correlation for most of the test chemicals, thus promising that the in-vitro test may be valuable to predict the eye irritating properties of chemicals once it is validated and accepted.
(2) Imidazole:
The in-vitro test result corresponded well with results from in-vivo tests using rabbits published in the literature.
Test condition: 12 laboratories tested 52 chemicals including imidazole in an in-vitro test system. Results for the individual chemicals was compared with results from in-vivo tests.
Test substance: 52 chemicals. Imidazole from Aldrich, unspecified purity, was included.
Conclusion: Promising in-vitro test for future use when validation is completed and the test system is accepted.
Reliability: (3) invalid
Unvalidated in-vitro test system.

17-JUL-2003 (113)

Type: other: Colipa Test -alternatives to the Rabbit eye irritation test

15-AUG-2000 (114)

Type: other: QSAR analysis for corrosivity

Remark: In a quantitative structure activity relationship analysis

Imidazol was considered to be essentially non corrosive. The analysis used 50 organic acids, 40 organic bases and 33 phenols and evaluated logP, molecular volume, pK, melting point and chemical substructures.

Test substance: Imidazole, no further data.

17-JUL-2003 (115) (116)

Type: other: cell transformation

Method: Mammalian cell transformation test. Method described by Marquardt et al. (1976) Cancer Res 36: 2065-2079

Result: Imidazole was negative in this test. For detail information see table below.

Treatment, mg/ml	Plating efficiency (summary)	Transformed foci/culture (summary)
Control	23	0/12
Imidazole		
0.1	27	0/11
1	23	0/14
2	16	0/14
4	8	0/16
MNNG, 0.025	15	18/13

Test condition: TEST SYSTEM
Logarithmically growing M2-C3H mouse fibroblasts (12th to 15th passage) were used. Cells were plated at 100 cells/petri dish for determination of cytotoxicity (duplicte) and at 1000 cells/plate for determination of the transformation rate (8 replicates).
ADMINISTRATION/DOSE LEVELS
After 24 hrs TS was added to the incubation medium during the 18-hr incubation period at dose levels of 0.1, 1, 2, and 4 mg/ml. Tests were conducted without metabolic activation. Negative and two positive controls N-methyl-N-nitro-N'-nitrosoguanidine) were included. Treatment period was 24 hours.
EVALUATION
The medium was changed after 24 h and the cells were allowed to grow for 2 weeks (determination of plating efficiency) or 8 weeks (determination of transformation rate). The cultures were fixed and stained with Giemsa and scored blind for type III foci (piled up, multilayered foci with criss-cross pattern at the edges, predominance of nuclear material).

Test substance: Imidazole >99% from Fluka

Reliability: (2) valid with restrictions
Meets scientific standards and is described in sufficient detail. No OECD guideline existent.

Flag: Critical study for SIDS endpoint

16-DEC-2003 (94)

Type: other: effects in humans

Remark: Imidazole salicylate was tested as a nonsteroidal anti-inflammatory drug in 67 human patients intolerant to Aspirin or related substances. Imidazole salicylate was found to be effective and essentially nontoxic.

Test substance: Imidazole salicylate, no further data.

18-SEP-2002 (117)

Type: other: in-vitrotoxicity testing using cell cultures

Remark: In vitro toxicity testing using cell cultures for predicting acute toxicity (LD50) for animal test reduction.

18-SEP-2002 (118)

Type: other: prediction of eye irritation by the test for permeability of mouse cornea

Remark: Imidazole was correctly identified as severe eye irritant by the test for permeabilty of mouse cornea. A drop of undiluted test substance was placed on the eye of a freshly killed mouse for 1 minute. After washing a drop of a fluorescent dye was placed on the cornea and the amount of corneal fluorescence was measured afterwards.
The test was not sufficiently validated in the given publication.

Test substance: Imidazole, no further data.

18-SEP-2002 (119)

Type: other: prediction of eye irritation with the in-vitro HET-CAM assay

Remark: Imidazole was correctly identified as severely irritant by the HET-CAM (hen egg test, chorionallantoic membrane) assay. Test results were compared with in vivo results of Gautheron et al. 1994. The HET-CAM test was performed as developed by Luepke and improved by using a microscopic examination.

Test substance: Imidazole from Aldrich, no further data.

18-SEP-2002 (120)

Type: other: testosterone secretion

Remark: Increasing doses(10-300 mg/kg) of imidazole were s.c. injected in adult rats (10 rats per group), samples of serum and testicular intestinal fluid (TIF) were collected 2 h later.

Result: Imidazole suppressed the two major regulating aspects of testicular function (testerone secretion and TIF formation) and can suppress LH secretion regulating systems in the pituitary in rats, supporting the hypothesis that imidazoles can suppress male reproductive function and fertility.
No microscopical examination of the testes was performed. Consequently there is no information about histopathological changes.

Test substance: imidazole: Sigma Chemical Company (St. Louis)

17-JUL-2003 (121)

Type: other: the EC/HO international validation study on alternatives to the Draize eye irritation test

Test substance: imidazole

17-JUL-2003 (122)

Type: other: tumor promotion

Remark: Mitotic activity was enhanced in primary rat hepatocytes incubated with imidazole in media high in calcium. Transition to S-phase was increased compared to control cells.
Tumor promoters increase DNA synthesis at low calcium concentrations.

12-JAN-2004 (123) (124)

Type: other: tumor promotion - chemoprevention

Method: Imidazole induced inhibition of thromboxan-synthetase was tested in chemical carcinogenesis.
Result: Tumor formation was not influenced by imidazole treatment.
Test condition: Sprague Dawley rats received single s.c. injections of N-Nitrosomethylurea. Imidazole was given at 1000 mg/kg in the diet from day 7

19-MAR-2004 (125)

6.1 Analytical Methods

6.2 Detection and Identification

7.1 Function

7.2 Effects on Organisms to be Controlled

7.3 Organisms to be Protected

7.4 User

7.5 Resistance

8.1 Methods Handling and Storing

Safe Handling: Breathing must be protected when large quantities are decanted without local exhaust ventilation.

Fire/Exp. Prot.: Prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy. Avoid dust formation. Dust can form an explosive mixture with air.

Storage Req.: Protect from acids and acid forming substances. Containers should be stored tightly sealed in a dry place.

Add. Information: Storage duration: 24 Months

Remark: Personal protective equipment

Respiratory protection:
Breathing protection if ventilation is inadequate.

Hand protection:
Chemical resistant protective gloves (EN 374) e.g. nitrile rubber (0.4 mm), chloroprene rubber (0.5 mm), polyvinylchloride (0.7 mm) and other

Manufacturers directions for use must be observed because of great diversity of types.

Eye protection:
Tightly fitting safety goggles (splash goggles) (EN 166)

Body protection:
Body protection must be chosen depending on activity and possible exposure, e.g. apron, protecting boots, chemical-protection suit (according to DIN-EN 465).

General safety and hygiene measures:
Avoid contact with the skin, eyes and clothing. Do not breathe dust.

Transport information

Land transport

ADR	Class	8
	Packaging group	III
	Substance no.	3263
	Designation of goods	CORROSIVE SOLID, BASIC, ORGANIC, N.O.S. (Contains: IMIDAZOLE)

RID	Class	8
	Packaging group	III
	Substance no.	3263
	Designation of goods	CORROSIVE SOLID, BASIC, ORGANIC, N.O.S. (Contains: IMIDAZOLE)

Inland waterway transport

ADNR	Class	8
	Item/Letter	55c)
	Packaging group	III
	Substance no.	3263
	Designation of goods	CORROSIVE SOLID, BASIC,

ORGANIC, N.O.S. (Contains: IMIDAZOLE)

Sea transport

IMDG/GGVSee Class 8
Packaging group III
UN-number 3263
Marine pollutant NO
Exact technical name CORROSIVE SOLID, BASIC, ORGANIC, N.O.S. (contains IMIDAZOLE)

Air transport

ICAO/IATA Class 8
Packaging group III
UN-number 3263
Exact technical name CORROSIVE SOLID, BASIC, ORGANIC, N.O.S. (contains IMIDAZOLE)

Flag: non confidential, Critical study for SIDS endpoint
08-APR-2003 (1)

8.2 Fire Guidance

Prot. Equipment: Wear self-contained breathing apparatus and chemical-protective clothing
Ext. Medium: water, dry extinguishing media, foam
Add. Information: Collect separately contamined extinguishing water, do not allow to reach sewage or effluent systems.

Flag: non confidential, Critical study for SIDS endpoint
08-APR-2003 (1)

8.3 Emergency Measures

Type: other: general advice

Remark: Immediately remove contaminated clothing. If danger of loss of consciousness, place patient in recovery position and transport accordingly. Apply artificial respiration if necessary. First aid personnel should pay attention to their own safety.
Flag: non confidential, Critical study for SIDS endpoint
13-DEC-2002 (1)

Type: injury to persons (skin)

Remark: Immediately wash thoroughly with plenty of water, apply sterile dressings, consult a skin specialist.
Flag: non confidential, Critical study for SIDS endpoint
13-DEC-2002 (1)

Type: injury to persons (eye)

Remark: Immediately wash affected eyes for at least 15 minutes under running water with eylids held open, consult an eye specialist.
Flag: non confidential, Critical study for SIDS endpoint
13-DEC-2002 (1)

Type: injury to persons (oral)

Remark: Rinse mouth immediately and then drink plenty of water, seek medical attention.
Flag: non confidential, Critical study for SIDS endpoint
13-DEC-2002 (1)

Type: injury to persons (inhalation)

Remark: Keep patient calm, remove to fresh air, seek medical attention.
Flag: non confidential, Critical study for SIDS endpoint
13-DEC-2002 (1)

Type: accidental spillage

Remark: Personal precautions:
Use breathing apparatus if exposed to vapours/dust/aerosol.
Avoid contact with the skin, eyes and clothing.

Environmental precautions:
Discharge into the environment must be avoided.

Methods for cleaning up or taking up:
Pick up with suitable appliance and dispose of.
Flag: non confidential, Critical study for SIDS endpoint
13-DEC-2002 (1)

8.4 Possib. of Rendering Subst. Harmless

8.5 Waste Management

Memo: other: must be dumped or incinerated in accordance with local regulations

Flag: non confidential, Critical study for SIDS endpoint
08-APR-2003 (1)

8.6 Side-effects Detection

8.7 Substance Registered as Dangerous for Ground Water

8.8 Reactivity Towards Container Material

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