FOREWORD INTRODUCTION

# *2-NITROANILINE*

## *CAS N°: 88-74-4*

# SIDS Initial Assessment Report
# For
# 13th SIAM
## (Bern, Switzerland, 6-9 November 2001)

**Chemical name:** 2-nitroaniline

**CAS no:** 88-74-4

**Sponsor Country:** France

**National SIDS Contact Point in Sponsor Country:**

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**History:**

The national peer review consisted of a presentation and critical discussion at a national panel of experts in toxicology and ecotoxicology from administration, university and industry and nominated by the ministry of environment. In parallel, a review was performed by the national institute on environmental and industrial risk (INERIS) by request from the ministry of environment. For this particular substance, only the verification of the most relevant underlying study reports or publications was performed.

**Testing completed :** toxicity towards algae (OECD GL 201)
Reprotoxicity/fertility (OECD GL 422)

**Comments:**

**Deadline for Circulation:** 14 September 2001

**Date of Circulation:** 14 September 2001

# SIDS INITIAL ASSESSMENT PROFILE

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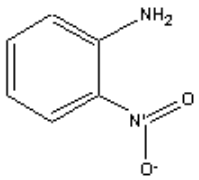
|---|---|
| **CAS No.** | 88-74-4 |
| **Chemical Name** | 2-nitroaniline |
| **Structural Formula** |  |

## RECOMMENDATIONS

The chemical is currently of low priority for further work.

## SUMMARY CONCLUSIONS OF THE SIAR

**Human Health**

The results of the published studies on 2-nitroaniline did not show significant increases of methemoglobin in animals except in the inhalation study. This difference with other isomers or inducers seems to be due to the difference of chemical reactivity of the nitro substitution in position 2 compared to other substitutions. 2-Nitroaniline is metabolised *in vitro* by rabbit liver microsomes to 4-amino-3-nitrophenol. 2-nitroaniline has been shown to have an oral LD50 value of 1838 mg/kg b/w in the rat, this is the only acute effect noted. It is not irritating to skin and to the eyes, and not sensitising. In oral repeated administration a NOEL of 50 mg/kg bw/day was determined from a 9 weeks study. The major treatment-related effects are clinical signs, but not methemoglobinemia, and weight loss. In a vapour inhalation 28 day assay a NOAEL was determined at 10 mg/m$^3$ in rats, due to slight methemoglobinemia and haematological effects seen at 90 mg/m$^3$.

2-nitroaniline was shown to be non-mutagenic in relevant bacterial studies. Nonetheless, a weak mutagenic influence was reported in some studies in which tests were performed on *S. typhimurium* strains TA98 and TA1538 in presence of Hamster S9 mix or with Flavin Mononucleotide activation. Investigations of general interaction with DNA on bacteria (*E.coli*) yielded negative results, as well as *in vitro* UDS tests and *in vivo* clastogenicity tests (micronucleus i.p.) or test on the alkaline elution behaviour of the DNA. In conclusion, 2-nitroaniline is not mutagenic.

In reproduction and developmental toxicological studies, the substance caused neither teratogenic nor fertility effects, but did cause developmental effects due to pups lethality at 450 mg/kg bw /day where a maternal body weight decrease occurred. The NOAEL for developmental effects was 150 mg/kg bw/day and the maternal NOAEL was set at 50 mg /kg bw in a study according to OECD TG 422.

**Environment**

2-nitroaniline has been found to be non-biodegradable, even in high inoculum concentration conditions. It therefore can be considered as persistent. The highest bioconcentration factor in fish was observed to be 8, leading to the conclusion that 2-nitroaniline does not significantly bioaccumulate.

The most valid and lowest E(L)C 50 found were a LC 50 (96 hours) in *Brachydanio rerio* of 19.5 mg/l, an EC 50 (24 hours) in *Daphnia magna* of 8.3 mg/l and an EC50 (growth rate, 72 hours) in *Selenastrum capricornutum* was > 100 mg/l. The lowest result is the EC 50 (24 hours) in *Daphnia magna.* Using an extrapolation factor of 1000, a PNEC of 0.008 mg/l can be estimated for the aquatic compartment.

**Exposure**

Estimated worldwide production of 2-nitroaniline is 20000 to 25000 tonnes/year. The production in the E.U. was 1000 to 5000 tonnes / year in 2000 in a unique site. The use in this region is non-dispersive, as an intermediate for synthesis in chemical industry. No other use could be documented in the EU. Nevertheless, the use in metal working fluids (<10%) and dyes (<1%) which can represent about 10% of the production volume were reported but not confirmed. 2-nitroaniline is an orange massive solid at room temperature, commercialised as flakes, or melted above 71 °C. It has a low vapour pressure at room temperature (0.00368 hPa at 25 °C) which reaches 1.33 hPa at 104 °C. So when melted, a potential exposure is possible by inhalation.

The water solubility of 2nitroaniline is 1170 mg/l at 20 °C and the measured log Pow is 1.85. Anilines are known to make covalent bonds to humic acids. Therefore 2-nitroaniline will distribute as such mainly to the water compartment in the environment, but could be covalently bound to sediments.

## NATURE OF FURTHER WORK RECOMMENDED

**Human Health and Environment**: The recommendation that this substance is not a priority for further work is based on the use of this substance exclusively as an intermediate in a closed system.

**Full SIDS Summary**

| CAS NO 88-74-4 | | SYSTEM – SPECIES | PROTOCOL | RESULTS |
|---|---|---|---|---|
| PHYSICO-CHEMICAL | | | | |
| 2.1 | Melting point | | | 69-71 °C |
| 2.2 | Boiling point | | | Decomposition at 280 °C |
| 2.3 | Density | | | 0.9015 at 25 °C |
| 2.4 | Vapour pressure | | | 0.00368 hPa at 25 °C |
| 2.5 | Partition coefficient | | | 1.85 |
| 2.6 | Water solubility | | | 1170 mg/l at 20 °C |
| 2.7 | Flash point | | | 167 °C |
| 2.10 | Explosive properties | | | Flakes, melted : no<br>Dusts : sensitive to ignition sources |
| ENVIRONMENTAL FATE AND PATHWAY | | | | |
| 3.1.1 | Photodegradation | | calculation | Rapid indirect photolysis (half-life 0.5 day) |
| 3.5 | Biodegradation | | OECD Guidelines 301 C, 302B | Not readily biodegradable<br>Not inherently biodegradable |
| 3.7 | Bioaccumulation | | Cyprinus carpio *Brachydanio rerio* | BCF = 2.1 – 4.9BCF = 8.1 |
| ECOTOXICOLOGY | | | | |
| 4.1 | Acute/prolonged toxicity to fish | Brachydanio rerio | OECD 203 | LC50 96h = 19.5 mg/l |
| 4.2 | Acute toxicity to aquatic invertebrates | Daphnia magna | OECD 202 | EC50 48h = 10-18 mg/l |
| 4.3 | Toxicity to aquatic plants e.g. algae | Selenastrum capricornutum | OECD 201 | EC50 > 100 mg/l<br>NOEC >= 100 mg/l |
| 4.4 | Toxicity to micro-organisms e.g. bacteria | Aerobic river bacteria | | EC50 24h = 34.7 mg/l |
| TOXICOLOGY | | | | |
| 5.0 | Metabolism | In vitro study on rabbit liver microsomes | Other | Main metabolite : 4-amino-3-nitrophenol |
| 5.1.1 | Acute Oral Toxicity | Rat | Other | LD50 = 1838 mg/kg |
| | | Rat | Other | LD50 = 3650 mg/kg |
| | | Mouse | Other | LD50 = 1290 mg/kg |
| 5.1.2 | Acute Inhalation Toxicity | No study available | | |
| 5.1.3 | Acute Dermal Toxicity | Rabbit | Other | LD50 > 20000 mg/kg |

| | | | | |
|---|---|---|---|---|
| 5.2.1 | Skin irritation/corrosion | Rabbit | Draize test | Not irritating |
| 5.2.2 | Eye irritation/Corrosion | Rabbit | Draize test | Not irritating |
| 5.3 | Sensitization | Guinea pig | OECD 406 Maximization test | Not sensitizing |
| 5.4.1 | Repeated Dose Toxicity by Inhalation | Rat (6 h / day / 4 week) | Other | NOAEL = 10 mg/m3 |
| 5.4.2 | Repeated Dose Toxicity by oral route | Rat (gavage 14 day) | Other | NOAEL = 100 mg/kg |
| | | Rat (gavage 9 weeks) | OECD 422 | NOEL = 50 mg/kg |
| 5.5.1 | GENETIC TOXICITY *IN VITRO* | | | |
| A. | Bacterial test (Gene mutation) | S. typhimurium TA98, TA100, TA1535, TA1537, TA1538 | Other (preincubation without incubation) | N (with activation)<br>N (without activation) |
| | | S. typhimurium TA98 | Other | P (with activation) |
| | | *S. typhimurium TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, D3052* | Other | N (with activation)<br>N (without activation) |
| | | S. typhimurium TA98, TA100, TA1535, TA1537, TA1538 | Other | N (with activation)<br>N (without activation) |
| | | *S. typhimurium TA97, TA102* | Other | N (with activation)<br>N (without activation) |
| | | *S. typhimurium TA98, TA100* | Other | N (without activation) |
| | | *S. typhimurium TA98, TA100* | Other | N (with activation)<br>N (without activation) |
| | | *S. typhimurium TA98, TA100, TA1535, TA1537, TA1538, TA97, TA2637* | Other | P (with activation)<br>P (without activation) |
| | | *S. typhimurium TA98* | Other | P (with activation Norharman S9) |
| | | *S. typhimurium G46, TA98, TA100, TA1535, TA1537, TA1538, C3076, D3052, E. coli WP2uvrA, WP2* | Other | N (with activation)<br>N (without activation) |
| | | *S. typhimurium TA98, TA100* | Other | TA100 : N (with and without activation)<br>TA98 : P (with activation hamster S9) |
| | | *S. typhimurium TA98, TA100, TA1535, TA1537, TA1538* | Other | N (with activation)<br>N (without activation) |
| | | *S. typhimurium TA153* | Other | P (with activation)<br>N (without activation) |

| | | | | |
|---|---|---|---|---|
| | | *S. typhimurium TA98, TA100* | Other | P (with activation and Flavin mononucleotide)<br>N (without activation) |
| | | *S. typhimurium TA98, TA100, E. coli WP2uvrA/pKM101* | Other | N (with activation)<br>N (without activation) |
| | | *S. typhimurium TA97, TA98, TA100, TA102* | Other | N (with activation)<br>N (without activation) |
| | | *S. typhimurium TA98, TA100* | Other | N (with activation)<br>N (without activation) |
| | | *S. typhimurium TA98, TA100, TA1535, TA1537, TA1538* | Other | N (with activation)<br>N (without activation) |
| | | *Bacillus subtilis H17, M45* | Other | P (without activation) |
| | | *E. coli WP2, WP2uvrA-* | Other | N (with activation)<br>N (without activation) |
| | | *E. coli WP2uvrA, WP2uvrA/pKM* | Other | P (with activation)<br>P (without activation) |
| B. | Non-bacterial *In Vitro* test (DNA damage and repair) | *E. coli WP2, WP67, CM871* | Other | P (with activation)<br>P (without activation) |
| | | *E. coli WP2, WP67, CM871* | Other | N (with activation)<br>N (without activation) |
| C. | Non-bacterial *In Vitro* test (Clastogenicity) | *Chinese hamster lung cell (CHL/IU)* | Other | P (with activation)<br>P (without activation) |
| D. | Non-bacterial *In Vitro* test (Unscheduled DNA synthesis) | *Rodent hepatocytes* | Other | N |
| | | *Rodent hepatocytes* | Other | N |
| | | *Rodent hepatocytes* | Other | N |
| 5.5.2 | GENETIC TOXICITY *IN VIVO* | | | |
| A. | Clastogenicity | Micronucleus test (mouse) | Other | N |
| | | Micronucleus test (mouse) | OECD 474 | N |
| B. | DNA damage | Alkaline elution (mouse) | Other | N |
| 5.7 | Carcinogenicity | No data available | | |
| 5.8 | Toxicity to reproduction | Rat | OECD 422 | NOAEL F0 and F1 = 50 mg/kg bw<br>LOAEL F0 and F1 = 150 mg/kg bw |
| 5.9 | Developmental toxicity / Teratogenicity | Rat | Other, but similar to OECD414 | NOAEL maternal = 100 mg/kg<br>NOAEL teratogenicity 300 mg/kg |
| | | | | |

| | | | | |
|---|---|---|---|---|
| | | Rat | Preliminary study before OECD 422 | NOAEL maternal = 200 mg/kg<br>NOAEL teratogenicity > = 400 mg/kg |
| 5.10 | Other data | | | |
| | Haematotoxicity | Rat (ip, 5h) | Other | MetHb at > 100 µmole/kg |
| | Haematotoxicity | | | |
| | QSAR DL50 | data are not taken in consideration in this evaluation | QSAR | Calculated LD50 = 783 mg/kg<br>Calculated LD50 = 500 mg/kg |
| 5.11 | Experience with human exposure | Some data are included in the IUCLID dossier | | |

Other : Protocol not according to the current guidelines
N : negative – P : positive

**SIDS INITIAL ASSESSMENT REPORT**

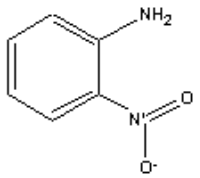
## 1. IDENTITY

Name (OECD): 2-nitroaniline

CAS number: 88-74-4

Molecular formula: $C_6H_6N_2O_2$

Structural Formula:




Molecular weight: 138.1

Other names: 2-nitro-1-aminobenzene
Orthonitroaniline
ONA

2-nitroaniline is an orange massive solid at room temperature, commercialised as flakes, or melted above 71 °C, with a purity > 99.6 %. The impurities are benzofurazane (< 0.2 %), nitrochlorobenzene (< 0.1 %) and water. The main physico-chemical properties are:

Vapour pressure : 0.00368 hPa at 25 °C, 1.33 hPa at 104 °C
Water solubility : 1170 mg/l at 20 °C
log Pow : 1.85
Henry'law constant : $5.9 \times 10^{-8}$ atm.m$^3$/mol,

## 2. GENERAL INFORMATION ON EXPOSURE

Estimated worldwide production of 2-nitroaniline is 20000 to 25000 tonnes/year. There are productions in Europe, in America and in Asia-Pacific. The production in the sponsor country (France) was 1000 to 5000 tonnes / year in 2000. The substance is produced at a unique site. Former producers in the EU (Bayer,Hoechst/ Clariant) contributed data to the current assessment. Information could not be retrieved from other worldwide producers.
The only use documented is non-dispersive, as an intermediate for synthesis in chemical industry. The main use (90%) is as an intermediate (delivered in molten form) for benzotriazoles used as anti-UV agents in plastics,. The others uses are also as an intermediate for dyes (around 5%, again molten form), and for metal cutting fluids (flakes) and around 1% as intermediate for pharmaceuticals (flakes).

### 2.1 Human exposure

2-Nitroaniline has a low vapour pressure at room temperature (0.00368 hPa at 25 °C) which reaches 1.33 hPa at 104 °C. So when melted, a potential exposure will be by inhalation.
However, workplace exposure can occur only in transferring the substance between containers, and during physical treatment (filtration / drying for flakes), as operations of production and chemical transformation are made in closed systems. This use as intermediate is the only known in Europe. Other potential uses at high temperature may lead to inhalation exposure.

### 2.2 Environmental fate

The water solubility of 2-nitroaniline is 1170 mg/l at 20 °C and the measured log Pow is 1.85. Therefore the water compartment will be one target compartment in the environment. The Henry constant is $5.9 \times 10^{-8}$ atm.m$^3$/mol, suggesting that the substance is not volatile from water. The EPIWIN Level II Fugacity model gave values of air : 0.5 %, water : 36.1 %, soil : 63.2 %, and sediment 0.1 %.
At production, a liquid effluent is released to the environment only after physico-chemical, then biological treatment. No gas emission occurs. At processing, which is exclusively chemical synthesis by less than 10 sites in the E.U. belonging to big Chemical Companies, the emission managing practices are essentially the same as at the production site.

***Photodegradation***

The indirect photodegradation in air was assessed using a calculation method, which was assigned validity 2. The half-life was 0.5 day with a concentration of OH radicals of $0.5 \times 10^6$ molecule/cm$^3$. 2-Nitroaniline emitted to the atmosphere in gaseous form would be rapidly degraded.

***Hydrolysis***

According to its stable chemical structure, 2-nitroaniline has no potential for hydrolysis.

***Biodegradation***

Three references were assigned validity 2. Two of them demonstrate that the substance is not readily biodegradable in tests in compliance with OECD ready biodegradability protocols. In another reference, a 10-20 % elimination after 3 hours has been observed in an inherent

biodegradability test, probably due to the adsorption of the test substance on sludge. Therefore 2-nitroaniline can be considered as not biodegradable.

***Adsorption/desorption in soils/sediments***

Anilines are known to form covalent bonds with humic compounds. Therefore an irreversible absorption on soils or sediments is supposed, the substance not being bioavailable as such. So no accumulation is expected in dwelling organisms.

***Bioaccumulation in fish***

Two references were assigned validity 1. Bioconcentration Factors of 8.1 in *Brachydanio rerio* and 2.1 -4.9 in *Cyprinus carpio* have been found. These results are consistent with the log Pow value of 1.85.

## 3. HUMAN HEALTH HAZARDS

**Preliminary remarks**

Reliability of the studies was evaluated using the criteria for reliability categories adapted from Klimisch et al. (1997) and Rosner (1994). Reliability is differentiated and thus classified into 4 categories/codes as described below. In this scoring system, studies conducted and reported according to internationally accepted test guidelines and in compliance with GLP have the highest grade of reliability and should be used as reference standards.

- *1 : Reliable without restriction*
  - 1a GLP guideline study (OECD, EC, EPA, FDA, etc …)
  - 1b Comparable to guideline study
  - 1c Test procedure in accordance with national standard methods (AFNOR, DIN, etc…)
  - 1d Test procedure in accordance with generally accepted scientific standards and described in sufficient detail.
- *2 : Reliable with restrictions*
  - 2a Guideline study without detailed documentation
  - 2b Guideline study with acceptable restrictions
  - 2c Comparable to guideline study with acceptable restrictions
  - 2d Test procedure in accordance with national standard methods with acceptable restrictions
  - 2e Study well documented, meets generally accepted scientific principles, acceptable for assessment
  - 2f Acceptable calculation method
  - 2g Data from handbook or collection of data
- *3 : Not reliable*
  - 3a Documentation insufficient for assessment
  - 3b Significant methodological deficiencies
  - 3c Unsuitable test system
- *4 : Not assignable*
  - 4a Abstract
  - 4b Secondary literature
  - 4c Original reference not yet available
  - 4d Original reference not translated (e.g Russian)
  - 4e Documentation insufficient for assessment

Studies selected for discussion are identified in the following tables by reliability 1 or 2 in the column “rel”. Other studies of validity 3 or 4 are only reported in the SIDS Dossier.

### 3.1 Effects on Human Health

#### 3.1.1 Mode of action of the chemical, toxicokinetics and metabolism

As the result of its aromatic nitro and amino grouping, 2-nitroaniline is described in the literature as a methemoglobin former, because both functional groups can be reduced or oxidised to reactive nitroso and hydroxylamine groups, respectively. By long exposure of animals, one can expect extramedullary hematopoeisis in the liver and spleen as the result of hypoxia (see results of Nair, 1983: repeated inhalation).

But in opposition with the 3/(meta)- or 4/(para)-nitroaniline, the results of the published studies showed neither consistent increase of methemoglobin nor extramedullary hematopoiesis. These

differences may be explained by the different chemical reactivity of these compounds by comparison with 2-nitroaniline. (see Shanin,1985; and Sergant, 1969).

Haematotoxicity / methemoglobinemia was detected by inhalation (90 mg/m$^3$/6 hours: 4 weeks) and methemoglobinemia reported by one intraperitoneal injection in rats at 14 mg/kg.
Among all the oral studies no indication of such an effect was detected and the structural differences in effects seen in acute toxicity, mutagenicity (Shahin, 1985)) or methemoglobinemia are supported by data on trifluoromethyl-anilines (Sergant, 1969) which indicate low effect for ortho-trifluoromethyl-anilines in the dog which is more sensitive than rat and than humans.
Following incubation of 2/(ortho)-nitroaniline with rabbit liver microsomes, 4-amino-3-nitrophenol was cited as the principal metabolite. Studies of pharmacokinetics *in vivo* are unavailable.

### 3.1.2 Acute toxicity

The acute toxicity studies conducted with 2-nitroaniline that could be checked are summarised in the following tables. None of these studies have been recently carried out, under national or international guidelines, and according to GLP.

***Acute oral toxicity***
From the 2 studies (Hoechst, 1973 and Vernot, 1977) assigned validity 2, the LD50 of 2-nitroaniline is probably around 1838 mg/kg, by the oral route, in the rat.
In human, this route represents a potential route of exposure.

Comparative data with the meta- and para- isomers indicate that ortho-nitroaniline has the lowest toxicity by this route.

**Table 3.1** – 2-nitroaniline – Acute oral toxicity

| Rel. | Species (strain), sex | Ref. (Year) | Protocol | Route of administration | Endpoint | Results (mg/kg) |
|---|---|---|---|---|---|---|
| 2 | Rat (ND) F | Hoechst (1973) | Other | Oral | LD50 | 1838 |
| 2 | Rat (SD) ND | Vernot (1977) | Other | Oral | LD50 | 3650 |
| 2 | Mouse (ND) ND | Vernot (1977) | Other | Oral | LD50 | 1290 |

Rel.: Reliability - ND: Not specified – SD: Sprague Dawley – F: female

***Acute inhalation toxicity***
No results from acute inhalation toxicity studies are available for 2-nitroaniline. But this route of potential intoxication is not relevant for man in the actual use as intermediate and due to its physical form. As 2-nitroaniline has a low vapour pressure, this makes human exposure only possible if used at high temperature in open systems.

***Acute dermal toxicity***
*Only one acute dermal toxicity study is available and assigned validity 2. This study conducted in the rabbit indicates an LD50 > 20 g/kg. So, no toxicity by dermal administration is expected in humans.*

### 3.1.3 Skin irritation/ corrosivity

Only one skin irritation study is available and assigned validity 2. This study conducted in the rabbit indicates that the product is not irritating for the skin of the rabbit, although the exposure was 24 hours and occlusive.

### 3.1.4 Eye irritation

Only one eye irritation study is available and assigned validity 2. This study conducted in the rabbit indicates that the product is not irritating for the eye.

#### *3.1.5 Sensitisation*

Only one skin sensitisation study is available and assigned validity 1. This study conducted in the guinea pig indicates that the product is not sensitising. Similar results were obtained in a patch test study performed on human patients hypersensitive to p-phenylene-diamine, though the reliability of this study is "not assignable", these results are supported by the results obtained in animals.

## 3.2 Repeated dose toxicity

Repeated dose toxicity studies with 2-nitroaniline are summarised in the following table (Table 3.2). One study was performed by inhalation (Nair, 1983), in the rat and assigned validity 2, and two by oral route (gavage) in the same species (Komsta, 1988 and Sisti, 2001). The duration of these studies was 4 weeks by inhalation, 2 weeks and 9 weeks by oral route and they were assigned validity 2 and 1.

### 3.2.1 Repeated dose toxicity by inhalation

In the whole body exposure study (Nair, 1983), the animals were exposed 6 hours per day for a period of 4 weeks (5 days a week) to 2-nitroaniline, at the concentrations of 0, 10 and 90 mg/m$^3$. As the maximum theoretical saturating vapour is around 20 mg/m$^3$, it can be considered that 90 mg/m$^3$ is a mixture of aerosol and vapour.

Increased tearing and nasal secretion as well as yellowing of the fir (whole body exposure) were reported in treated groups.

No treatment effects were observed on the body weight gain of the rats and on the major organs examined (macroscopic and histological examinations), in particular no effects on testicles.

At 90 mg/m$^3$, the only effects seen were a slight increase of the methemoglobin level and the hematocrit value, as well as a marginal reduction of the leukocytes and the segmented neutrophil counts. No effects were reported at 10 mg/m$^3$.

So in conclusion, by inhalation the NOAEL is 10 mg/m$^3$.

### 3.2.2 Repeated dose toxicity by oral route

In a 14 days repeated dose toxicity study (Komsta, 1988) by oral route, the product was administered to the animals by gavage, at 0, 1, 10 and 100 mg/kg.

No treatment effects were observed on the behaviour, the body weight gain of the rats and the major organs examined (macroscopic and histological examinations), in particular no effects on testicles. No effects of toxicological importance were seen in this study, whatever the administered dose level.

The NOAEL in this study was >= 100 mg/kg.

In the second study performed according to the OECD guideline 422 (Sisti, 2001), male and female rats were treated orally by gavage with 450, 150 and 50 mg/kg bw/day in PEG 400. There was

minimal toxic effect at 450-mg/kg bw/day. At lower doses the body weight gain was the only sign found. No effect was noted on histology.
The NOEL is 50 mg/kg and the LOAEL is 150 mg/kg bw/day.

*In conclusion*, by oral route on 9 weeks the NOEL was established at 50 mg/kg bw/day due to some decrease in body weight gain.

### 3.2.3 Repeated dose toxicity by other routes

No data are available on repeated dose toxicity studies by dermal or other routes for 2-nitroaniline.

Table 3.2– 2-nitroaniline – Repeated dose toxicity studies

| Rel. | **Ref. (Year)** | **Species** | **Route of administration** | Protocol | **Duration Frequency** | **Administration** | Doses | Endpoints | Results |
|---|---|---|---|---|---|---|---|---|---|
| 2 | Nair (1983) | Rat | Inhalation | Other | 4 weeks<br>6h/d, 5d/week | Whole body | 0, 10, 90 $mg/m^3$ | Behaviour<br>Observation<br><br>BW<br>MetHb<br>Histopathology<br>NOAEL | NS<br>I; tearing and nasal secretions<br>NS<br>I; 90 $mg/m^3$<br>NS<br>10 $mg/m^3$ |
| 2 | Komsta (1988) | Rat | Oral route | Other | 14 d<br>7d/week | Gavage | 0, 1, 10, 100 mg/kg b/w | Behaviour<br>BW<br>Haematology<br>Biochemistry<br>Histopathology<br>NOAEL | NS<br>NS<br>NS<br>NS<br>NS<br>100 mg/kg bw |
| 1 | Sisti (2001) | Rat | Oral route | 422 | 9weeks<br>7days/week | Gavage | 0, 50, 150 and 450 mg/kg bw/d | Behaviour<br>BW<br>Histopathology<br>NOEL | NS<br>S Male + /-Female<br>NS<br>50 mg/kg bw |

*Rel.: Reliability – b/w or BW: Body Weight – NS: No alteration – MetHb: methemoglobinemia – I: increase*

## 3.3 Genetic toxicity

### 3.3.1 Genetic toxicity in vitro

There are 18 reported data in this section, 12 being assigned validity 1 or 2. Only the latter will be taken into consideration for analysis of *in vitro* genotoxicity of 2-nitroaniline.
In the *Ames* test, there are 2 reports of validity 1 (Shahin, 1985 and Shimizu et al., 1986) which indicate negative effect. They are supported by 3 reports of validity 2 (Chiu, 1977, Blakey, 1994 and Assmann, 1997). But according to the strain and the activation system (S9 mix) used, 2-nitroaniline has been shown to be negative without and positive with the S9 mix of hamster with Flavin Mononucleotide, in the TA98 strain (Le, 1985 and Dellarco, 1989) or in the TA1538 strain (Garner, 1977). These results are then in contradiction with the other ones, but one must stress that the study of Shahin using different S9 indicates that S9 from hamster does not behave like that form other mammals including human.
Regarding *Escherichia co li* gene mutation tests (or DNA repair test) on *Escherichia coli WP2, WP67, CM871*, 3 studies were reported and the one with validity 2 (Thompson, 1983) showed negative results, as well as the 2 others of validity 3 ( De Flora, 1984; Kawaï, 1987).
On *mammalian cells,* 3 studies of validity 2 are reported. One positive result was observed *in vitro* on clastogenicity in Chinese hamster lung cells (CHL/IU, Matsushima, 1999, validity 2) at very high cytotoxic (not reported) doses. On the other hand negative results are reported in the Unscheduled DNA synthesis in 2 rodent hepatocytes assays (validity 2; Yoshimi, 1988 and Thompson, 1983). It is concluded that in normal conditions the substance is not mutagenic *in vitro* .

### 3.3.2 Genetic toxicity in vivo

*In vivo*, 2 tests were performed: one micronucleus test, via intraperitoneal route, with validity 2 (Cesarone, 1993) and a DNA damage test Alkaline elution with validity 1 (Herbold,1982) were negative. They do not confirm some of the positive results seen *in vitro*.
It is concluded that 2-nitroaniline is not genotoxic *in vivo*, even by i.p. route which is not a human route of exposure. These results *in vivo* support the negative results obtained *in vitro*.

In conclusion, 2-nitroaniline was shown to be non-mutagenic .

Table 3.3– 2-nitroaniline – Genetic toxicity

| Rel. | Ref. (year) | System – Secies | Protocol | Results |
|---|---|---|---|---|
| In vitro Tests | | | | |
| 1 | Shahin (1985) Shimizu (1986) | *S. typhimurium TA98, TA100, TA1535, TA1537, TA1538* | Preincubation without incubation GL 401 | N (with activation) N (without activation) |
| 2 | Thompson (1983) | *S. typhimurium TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, D3052* | Other #GL 401 | N (with activation) N (without activation) |
| 2 | Chiu (1977) | *S. typhimurium TA98, TA100,* | Other #GL 401 | N (without activation) |
| 2 | Le (1985) | S. typhimurium TA98, TA100 | Other #GL 401 | TA100 : N TA98 : P (with hamster S9) N others |
| 2 | Garner (1977) | S. typhimurium TA1538 | Other #GL401 | P (with activation) N (without activation) |
| 2 | *Dellarco (1989)* | S. typhimurium TA98, TA100 | Other #GL401 | P (with activation / FM) N (without activation) |
| 2 | *Blakey (1994)* | *S. typhimurium TA97, TA98, TA100, TA102* | Other #GL401 | N (with activation) N (without activation) |
| 2 | *Assmann (1997)* | S. typhimurium TA98, TA100 | Other #GL401 | N (with activation) N (without activation) |
| 2 | *Thompson (1983)* | Escherichia coli WP2, WP2uvrA- | Other#402 | N (with activation) N (without activation) |
| 2 | *Matsushima (1999)* | Micronucleus in Chinese hamster lung cell (CHL/IU) | Other | P (with activation) P(without activation) |
| 2 | *Yoshimi (1988)* | UDS in rodent hepatocytes | Other #GL | N |
| 2 | *Thompson (1983)* | UDS in rodent hepatocytes | Other #GL | N |
| *In vivo Tests* | | | | |
| 2 | *Cesarone (1993)* | Micronucleus test (mouse) | OECD 474 | N |
| 1 | *Herbold (1982)* | DNA Damage Alkaline elution (mouse) | Other | N |

Rel.: reliability – Other: Protocol not according to the current guidelines
N: negative – P: positive - FM: Flavin Mononucleode

## 3.4 Carcinogenicity

No carcinogenicity studies are available after oral, dermal or inhalation exposure to 2-nitroaniline.

## 3.5 Toxicity to reproduction and developmental toxicity/teratogenicity

### 3.5.1 Toxicity to reproduction/Fertility.

A reproduction fertility study (Sisti, 2001) was performed according to the OECD 422 Guideline with Sprague-Dawley rats by gavage in PEG 400 at 0, 50, 150 and 450 mg/kg bw/day. Males were treated for 9 weeks, starting 4 weeks before mating, female were treated as well up to 4 days after delivery.

**Parental results:**

- Clinical observation: the only signs related to treatment were piloerection, salivation and matted fur observed after treatment (high-dose group).
  Body weights: significant reduction in body weight were observed at several weighing times in the high- and mid-dose groups (males and females: 5-6%) during the treatment and terminal body-weight was observed in high-dose males.
- Some high dose females on gestation day 20 and on day 4 post-partum lost weight (up to 25%) or did not gain weight compared to controls. This had a direct effect on pups' mortality.
- Organ weights: No differences were observed in absolute and relative organ weights of male parents.
- Macroscopic and microscopic observations of parental generation: macroscopic and microscopic examinations of all organs, including spermatogenic cycle, did not reveal any treatment-related effects
- Reproductive parameters were unaffected by treatment: the copulatory and fertility index, as well as the pre-coital intervals were not affected by treatment. Implantation and pre-birth losses were unaffected by treatment.
  F1 results:
- litter viability and growth and sex-ratios: Litter size and litter weight were statistically significantly reduced on day 4 post-partum in the high-dose group when compared to controls, while a statistically significant increase in cumulative loss was also observed in the same group. In addition, a statistically significant increase in male pup death was observed in the high-dose group compared to controls.
- Necropsy findings in decedent pups: the findings observed at necropsy in decedent pups were similar in the control and the treated groups, at day 4 post-partum with the exception of 2 pups each in the mid- and high-dose groups that showed abnormal size of the median lobe of the liver in association with an abnormal area and abnormal colour.

It is concluded that all reproductive parameters were unaffected by treatment at 450 mg/kg and the general toxicity NOAEL is = 50 mg/kg bw / day for F0 and F1 generations.

### 3.5.2 Developmental toxicity/ teratogenicity

Two studies were assigned validity 2 (Farr, 1984, 1985) and one validity 1 (Sisti, 2001).
A developmental toxicity/teratogenicity study, close to the current guideline OECD 414, was performed in the rat after a preliminary study.

In the study by Farr (1985), the animals were treated by gavage at 0, 100, 300 and 600 mg/kg b/w of 2-nitroaniline, in oil vehicle, from day 6 to day 15 of the gestation.
Under these conditions, no effects were observed on the fetuses at doses without effects on the dams. The endpoints given were:

NOAEL for maternal toxicity was 100 mg/kg b/w based on the effects on body weights and a decrease of the food consumption at higher dose levels.
NOAEL for fetal toxicity (embryotoxicity and teratogenicity) was 300 mg/kg b/w.

Before performing a full OECD 422 study, a preliminary study was performed by Sisti (2001) according to the criteria of OECD TG 414, the maternal and developmental toxicity of 2-nitroaniline were assessed in the rat during gestation:
2-Nitroaniline was administered daily by gavage to females from Day 0 to Day 19 of gestation at doses of 0, 100, 200 and 400 mg/kg/day. Control animals received the vehicle alone (Polyethylene glycol 400). The females were killed on gestation Day 20 and subjected to a post-mortem examination.
The number of corpora lutea, weight of intact gravid uterus, number and distribution of live fetuses, number and distribution of intra-uterine deaths, and individual fetal weight and sex were determined. All fetuses were examined externally.
Matted fur and piloerection were the only clinical signs observed in the high-dose group. Group mean body weight and body weight gain were unaffected by treatment. All females were pregnant and had live fetuses on gestation Day 20. Litter data and sex ratios did not show any treatment-related effects.
There were no differences in uterus and corrected body weight between the control and the treated groups. Macroscopic examinations in females and fetal examinations did not show any treatment-related effects.
In this study, the NOAEL for maternal toxicity was 200 mg/kg b/w and for the fetal toxicity ≥ 400 mg/kg b/w.

It can be concluded from this relevant study that maternal toxicity NOAEL is 200 mg/kg bw/day while the NOAEL for fetal and development toxicity is higher than 400 mg/kg bw day.

### 3.6 Endpoints for Human health:

| | | | |
|---|---|---|---|
| Acute oral toxicity | | LD50 | 1838 mg/kg bw |
| Repeated Inhalation | (4 weeks) | NOAEL | 10 mg/m3 |
| Repeated oral toxicity / | | NOAEL | 50 mg/kg bw |
| Reprotoxicity (9 weeks) for F0 and F1 | | NOAEL | 50 mg/kg bw |
| Developmental | Maternal | NOAEL | 200 mg/kg bw |
| | Feta1 | NOAEL | 400 mg/kg bw |
| | Teratogenicity | NOAEL | 400 mg/kg bw |

**Initial Assessment for Human exposure:**

As an intermediate prepared in molten form or flakes and filled in drums or tanks, the oral route does not represent an important route of exposure. No other acute effect is expected. At liquid stage and at high temperature exposure to vapour may represent a hazard if no precaution is taken (ventilation, aspiration for example). This does not seem to be important in the use as an intermediate, but could be for other uses.

# 4. HAZARDS TO THE ENVIRONMENT

## 4.1 Aquatic effects

### Acute toxicity in fish

Only one reference was assigned validity 1. The study was performed according to the OECD Guidelines 203 (1984) in a 96 hours semi-static test on *Brachydanio rerio*, resulting in an $LC_{50}$ 96 h of 19.5 mg/l.
Two other references were assigned validity 2. One study was performed according to the OECD Guideline 203 under GLP, but the concentrations were not measured. *Brachydanio rerio* were exposed 96 hours in static conditions to test substance. The obtained LC50 ranged from 10 to 22 mg/l.
Another one was performed in *Cyprinus carpio* according to a protocol in compliance with the main criteria of OECD TG 203. The result was a LC50 96h of 16.2 mg/l.
The validity 2 results have to be taken with precaution, as no analytical control was performed and no substance purity data was given. However, as 2-nitroaniline is neither biodegradable nor volatile, nor particularly adsorbable, and the LC50 values look consistent with values obtained with those obtained in other taxa, these values can be considered as acceptable for the hazard assessment.
One published test result indicated a 48h-LC50 of 1.66 mg/l for *Carassius auratus*. This test result was considered to be non-valid. When the fish LC50 data set of 2-nitroaniline is analysed, it can be observed that one of the four validated value is one order of magnitude below this of the other values :

- LC50 96h *Brachydanio rerio* = 19.5 mg/l (assigned Validity 1)
- LC50 48h *Carassius auratus* = 1.66 mg/l (assigned Validity 2)
- LC50 96h *Cyprinus carpio* = 16.2 mg/l (assigned Validity 2)
- LC50 96h *Brachydanio rerio* = 10-22 mg/l (assigned Validity 2)

Several reasons are leading to invalidate the *Carassius auratus* LC50 value :

a) it is one order of magnitude below this of the 3 other values, and particularly than the only Validity 1 value.
b) The purity announced in the publication is > 95 %, which lets the opportunity of occurrence of a toxic impurity. A lack of data on identification and quantification of impurities is a major factor of invalidation of a study. However, if such a study result is consistent with most of the validated results, or if doubtful results are within the same range of magnitude, they can validate each other, because the probability to have got the exact value is higher. On the contrary, if one result is within a different range from other values, the lack of data on impurities becomes very important, because there it is likely that the abnormal toxicity value is due to an unknown impurity.
c) *Carassius auratus* is a Cyprinides, like the 3 other fish and its sensitivity is not supposed to be very different.
d) In the same publication, a LC50 48h on 4-nitroaniline has been found to be 1.2 mg/l. However, the fish toxicity data found in the IUCLID file for 4-nitroaniline are :

- LC50 96h *Pimephales promelas* = 106 mg/l
- LC50 96h *Brachydanio rerio* = 89 mg/l
- LC50 48h *Leuciscus idus* = 35 mg/l
- LC50 96h *Oryzias latipes* = 84 mg/l
- LC50 48h *Salmo gairdneri* = 28-56 mg/l

As the substance is neither biodegradable nor adsorbable nor volatile, an underestimation of toxicity due to loss of substance is unlikely. Moreover, the IUCLID data set is rather consistent, and the

value found in this publication is clearly out of this range. This confirms the hypothesis that the nitroaniline samples tested were containing some impurities more toxic than the substance itself.
The result is therefore considered as invalid.
The LC50 96h retained was therefore 19.5 mg/l because of best reliability.

**Acute toxicity in invertebrates**

Three references describing test results with *Daphnia magna* were assigned validity 2. One test was performed during 48h according to the OECD Guideline 202 and under GLP, without indication of the test conditions (static, semi-static, dynamic). Concentrations were not measured, but the substance was of known origin. The obtained $EC_{50}$ (48 h) ranged from 10 to 18 mg/l.
Another EC50 48h found was 10.5 mg/l and an EC50 24h in another test was 8.3 mg/l. No analytical control was performed in these two tests and substance purity was given only in the latter. However, as 2-nitroaniline is neither biodegradable nor volatile, nor particularly adsorbable, and the EC50 value looks consistent with values obtained with those obtained in other taxa, this value can be considered as acceptable for the hazard assessment.
The value retained for the PNEC calculation is 8.3 mg/l.

**Toxicity in aquatic plants / algae**

One result was assigned validity 2, because no analytical control was performed and substance purity was not given. It was obtained in a test performed according to general rules given in the OECD guideline 201, in *Scenedesmus obliquus*: the EC50 96h for growth rate was 64.6 mg/l.
A study recently performed on a sample of high purity (> 99.6 %) in *Selenastrum capricornutum*, according to GLP and OECD guidelines, was assigned therefore validity 1. No inhibition was observed in a limit test so the EC50 72h was > 100 mg/l, and NOEC >= 100 mg/l. As being of best validity, this result was retained for PNEC calculation.

**Toxicity in micro-organisms**

Four references were assigned validity 2. One result was obtained in *Photobacterium phosporeum* luminescence, in a "Microtox" type test. This kind of result cannot be used for hazard assessment in micro-organisms.
An EC50 24h for growth rate in river aerobic bacteria was found to be 34.7 mg/l, an EC50 3d in methanogenic bacteria was found to be 1.9 mg/l, and an EC50 40h in a protozoan : *Tetrahymena pyriformis*, was 115 mg/l.
*For assessing hazards in an aerobic wastewater treatment plants, an EC50 of 34.7 mg/l in bacteria can be retained, as a lower toxicity towards protozoans is shown. Hazards to anaerobic treatment plants can be assessed with the value EC50 3d of 1.9 mg/l.*

## 4.2 Terrestrial effects

The only terrestrial toxicity test reported on 2-nitroaniline was a test in birds that was assigned validity 3. The test was not performed according to standardised Guidelines and few details were given concerning the test conditions.
The test substance was administered by gavage. The obtained $LD_{50}$ was 750 mg/kg for *Agelaius phoenicus* and *Coturnix coturnix* and > 1000 mg/kg for *Sturnus vulgaris*.

### 4.3 PNEC derivation

The L (E) C50 selected for a PNEC derivation were:
1) $LC_{50}$ 96 h (*Brachydanio rerio* ) = 19.5 mg/l
2) $EC_{50}$ 48 h (*Daphnia magna*) = 8.3 mg/l
3) $EC_{50}$ (*Selenastrum capricornutum* growth rate) > 100 mg/l

As the most sensitive species in data assigned with validity 2 or 1 is *Daphnia magna* and no chronic test result is available in this species, the PNEC is derived by applying an assessment factor of 1000 to the EC50 for Daphnia :
PNEC aqua = 0.0083 mg/l.

**Initial Assessment for the Environment**

2-Nitroaniline has been found to be non-biodegradable. It does not bioaccumulate significantly. The most valid and lowest E(L)C 50 found were a 96h - LC 50 in *Brachydanio rerio* of 19.5 mg/l, a 24h EC 50 in *Daphnia magna* of 8.3 mg/l and a 96h - EC50 (growth rate) of *Scenedesmus obliquus* was 64.6 mg/l. A PNECaqua of 0.008 mg/l was derived based on these data. Provided that the substance is used as a chemical intermediate only, the substance is currently of low priority for further work. If any other use became apparent, an in-depth risk assessment would be warranted.

## 5. CONCLUSIONS AND RECOMMENDATIONS

The chemical is currently of low priority for further work. The recommendation is based on the use of this substance exclusively as an intermediate in a closed system

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# *I U C L I D Data Set*

| | | |
|---|---|---|
| **Existing Chemical** | : | ID: 88-74-4 |
| **CAS No.** | : | 88-74-4 |
| **EINECS Name** | : | 2-nitroaniline |
| **EC No.** | : | 201-855-4 |
| **TSCA Name** | : | Benzenamine, 2-nitro- |
| **Molecular Formula** | : | C6H6N2O2 |

**Producer related part**

| | | |
|---|---|---|
| **Company** | : | RHODIA Services/Direction Product Stewardship |
| **Creation date** | : | 21.12.2001 |

**Substance related part**

| | | |
|---|---|---|
| **Company** | : | RHODIA Services/Direction Product Stewardship |
| **Creation date** | : | 21.12.2001 |
| **Status** | : | |
| **Memo** | : | |
| **Printing date** | : | 11.02.2003 |
| **Revision date** | : | |
| **Date of last update** | : | 11.02.2003 |
| **Number of pages** | : | 1 |
| **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, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS |

### 1.0.1 APPLICANT AND COMPANY INFORMATION

| | | |
|---|---|---|
| **Type** | : | cooperating company |
| **Name** | : | Rhodia Organique |
| **Contact person** | : | M. Jean-François Clabaut |
| **Date** | : | 26.04.2001 |
| **Street** | : | Usine de Mulhouse-Dornach |
| **Town** | : | 68059 Mulhouse |
| **Country** | : | France |
| **Phone** | : | +33 3 89 32 60 25 |
| **Telefax** | : | +33 3 89 32 13 63 |
| **Telex** | : | |
| **Cedex** | : | |
| **Email** | : | |
| **Homepage** | : | |
| **Source** | : | Rhodia Recherches Saint Fons |
| **26.04.2001** | | |

### 1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

| | | |
|---|---|---|
| **Type** | : | manufacturer |
| **Name of plant** | : | Usine de Mulhouse-Dornach |
| **Street** | : | |
| **Town** | : | |
| **Country** | : | |
| **Phone** | : | |
| **Telefax** | : | |
| **Telex** | : | |
| **Cedex** | : | |
| **Email** | : | |
| **Homepage** | : | |
| **14.01.2002** | | |

### 1.0.3 IDENTITY OF RECIPIENTS

### 1.0.4 DETAILS ON CATEGORY/TEMPLATE

### 1.1.0 SUBSTANCE IDENTIFICATION

### 1.1.1 GENERAL SUBSTANCE INFORMATION

| | | |
|---|---|---|
| **Purity type** | : | |
| **Substance type** | : | organic |
| **Physical status** | : | solid |
| **Purity** | : | >= 99.6 % w/w |
| **Colour** | : | |
| **Odour** | : | |
| **Source** | : | Rhodia Recherches Saint Fons |
| **18.04.2001** | | |

### 1.1.2 SPECTRA

### 1.2 SYNONYMS AND TRADENAMES

**2-NITRO-1-AMINOBENZENE**
**Source** : Rhodia Recherches Saint Fons
**18.04.2001**

**ONA**
**Source** : Rhodia Recherches Saint Fons
**29.07.1996**

**ORTHONITROANILINE**
**Source** : Rhodia Recherches Saint Fons
**29.07.1996**

### 1.3 IMPURITIES

**Purity** :
**CAS-No** : 273-09-6
**EC-No** :
**EINECS-Name** : BENZOFURAZANE
**Molecular formula** :
**Value** : <= .2 % w/w
**Source** : Rhodia Recherches Saint Fons
**29.07.1996**
**Purity** :
**CAS-No** : 88-73-3
**EC-No** : 201-854-9
**EINECS-Name** : 1-chloro-2-nitrobenzene
**Molecular formula** :
**Value** : <= .1 % w/w
**Source** : Rhodia Recherches Saint Fons
**23.05.2001**

### 1.4 ADDITIVES

### 1.5 TOTAL QUANTITY

**Quantity** : 1000 - 5000 tonnes produced in 2000
**Remark** : Worldwide annual production is estimated to: 20 000 to 25 000 tonnes / year

Producers in :
European Union
Japan
India
China
USA

**Source** : Rhodia Recherches Saint Fons
**25.06.2002**

### 1.6.1 LABELLING

| | | |
|---|---|---|
| **Labelling** | : | as in Directive 67/548/EEC |
| **Specific limits** | : | no |
| **Symbols** | : | T, , , |
| **Nota** | : | C, , |
| **R-Phrases** | : | (23/24/25) Toxic by inhalation, in contact with skin and if swallowed<br>(33) Danger of cumulative effects<br>(52/53) Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment |
| **S-Phrases** | : | (28) After contact with skin, wash immediately with plenty of ...<br>(36/37) Wear suitable protective clothing and gloves<br>(45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)<br>(61) Avoid release to the environment. Refer to special instructions/Safety data sets |
| **Remark** | : | (1/2) : keep locked up and out of reach of children.<br>This phrase is mentioned in 21st ATP, but not applicable to this substance which is not in contact with the public.<br>Annex I entry : 612-012-00-9 for o-, m- and p-nitroaniline.<br>21st ATP |
| **Source** | : | Rhodia Recherches Saint Fons |
| **23.04.2001** | | |

### 1.6.2 CLASSIFICATION

| | | |
|---|---|---|
| **Classified** | : | as in Directive 67/548/EEC |
| **Class of danger** | : | toxic |
| **R-Phrases** | : | (23/24/25) Toxic by inhalation, in contact with skin and if swallowed<br>(33) Danger of cumulative effects<br>(52/53) Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment |
| **Specific limits** | : | |
| **Remark** | : | Annex I entry : 612-012-00-9<br>21st ATP |
| **Source** | : | Rhodia Recherches Saint Fons |
| **18.04.2001** | | |

### 1.6.3 PACKAGING

## 1.7 USE PATTERN

| | | |
|---|---|---|
| **Type of use** | : | type |
| **Category** | : | Non dispersive use |
| **Source** | : | Rhodia Recherches Saint Fons |
| **29.07.1996** | | |
| **Type of use** | : | industrial |
| **Category** | : | Chemical industry: used in synthesis |
| **Source** | : | Rhodia Recherches Saint Fons |
| **29.07.1996** | | |
| **Type of use** | : | use |
| **Category** | : | Intermediates |
| Source | : | Rhodia Recherches Saint Fons |
| 18.04.2001 | | |

### 1.7.1 DETAILED USE PATTERN

**1.7.2 METHODS OF MANUFACTURE**

**1.8 REGULATORY MEASURES**

**1.8.1 OCCUP ATIONAL EXPOSURE LIMIT VALUES**

**1.8.2 ACCEPTABLE RESIDUES LEVELS**

**1.8.3 WATER POLLUTION**

**1.8.4 MAJOR ACCIDENT HAZARDS**

**1.8.5 AIR POLLUTION**

**1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES**

**1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS**

**1.9.2 COMPONENTS**

**1.10 SOURCE OF EXPOSURE**

Remark : At workplace : only in transferring the substance between contaners, and during phyical treatment (filtration / drying)
Source : Rhodia Recherches Saint Fons
23.04.2001

Remark : Liquid effluent released only after physico-chemical, then biological treatment.
Source : Rhodia Recherches Saint Fons
18.04.2001

**1.11 ADDITIONAL REMARKS**

**1.12 LAST LITERATURE SEARCH**

**Type of search** : Internal and External
**Chapters covered** : 3, 4, 5
**Date of search** : 10.10.2000
**02.01.2002**

**1.13 REVIEWS**

## 2.1 MELTING POINT

| | | |
|---|---|---|
| **Value** | : | = 69 - 71 °C |
| **Sublimation** | : | |
| **Method** | : | |
| **Year** | : | 1983 |
| **GLP** | : | |
| **Test substance** | : | |
| **Source** | : | Rhodia Recherches Saint Fons |
| **23.04.2001** | | (1) |

## 2.2 BOILING POINT

| | | |
|---|---|---|
| **Value** | : | = 280 °C at |
| **Decomposition** | : | yes |
| **Method** | : | other: DTA |
| **Year** | : | 1989 |
| **GLP** | : | no data |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | The temperature given is decomposition temperature |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | 3 K/min |
| **Test substance** | : | Production from Hoechst |
| **Reliability** | : | (2) valid with restrictions |
| **23.04.2001** | | (2) |
| **Value** | : | = 280 °C at |
| **Decomposition** | : | yes |
| **Method** | : | other |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Method** | : | Differential Thermic Analysis (4°C / min) |
| **Result** | : | Decomposition enthalpy : 490 cal/g |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Current protocol followed on known substance, but not in GLP. |
| **23.04.2001** | | |
| **Value** | : | = 284 °C at |
| **Method** | : | calculation |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Data from handbook |
| **23.04.2001** | | (3) |

## 2.3 DENSITY

| | | |
|---|---|---|
| **Type** | : | relative density |
| **Value** | : | = .9015 at 25 °C |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Data from handbook |
| **23.04.2001** | | (4) |

| | | |
|---|---|---|
| **Type** | : | density |
| **Value** | : | = 1250 kg/m3 at 80 °C |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions |
| **23.04.2001** | | (5) |

### 2.3.1 GRANULOMETRY

## 2.4 VAPOUR PRESSURE

| | | |
|---|---|---|
| **Value** | : | = .00368 hPa at 25 °C |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Data from Handbook |
| **23.04.2001** | | (6) |
| **Value** | : | = 1.33 hPa at 104 °C |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Data from Handbook |
| **23.04.2001** | | (7) |

## 2.5 PARTITION COEFFICIENT

| | | |
|---|---|---|
| **Partition coefficient** | : | |
| **Log pow** | : | 1.78 at °C |
| **pH value** | : | |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (4) not assignable<br>Citation |
| **23.05.2001** | | (8) |
| **Partition coefficient** | : | |
| **Log pow** | : | = 1.8 at °C |
| **pH value** | : | |
| **Method** | : | other (calculated) |
| **Year** | : | 1991 |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | Leo, Hansch: Medchem Software CLOGP3, Release 3.42, Pomona College, Clermont CA |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Recognised calculation method |
| **25.06.2001** | | (9) |
| **Partition coefficient** | : | |
| **Log pow** | : | = 1.85 at °C |
| **pH value** | : | |
| **Method** | : | other (measured): no data |
| **Year** | : | 1985 |
| **GLP** | : | no data |

| | | |
|---|---|---|
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Cited in official report (BUA) |
| **25.06.2001** | | (10) |
| **Partition coefficient** | : | |
| **Log pow** | : | = 2.02 at °C |
| **pH value** | : | |
| **Method** | : | other (calculated) |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |
| **Method** | : | KOWWIN, Syracuse Research Corporation |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Recognised calculation method |
| **25.06.2001** | | |

### 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

| | | |
|---|---|---|
| **Solubility in** | : | Water |
| **Value** | : | = 7.5 g/l at 50 °C |
| **pH value** | : | |
| **concentration** | : | at °C |
| **Temperature effects** | : | |
| **Examine different pol.** | : | |
| **pKa** | : | at 25 °C |
| **Description** | : | |
| **Stable** | : | |
| **Deg. product** | : | |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Test not made in GLP, on the substance of highest purity. |
| **27.01.2003** | | (11) |
| **Solubility in** | : | Water |
| **Value** | : | = 1.47 g/l at 25 °C |
| **pH value** | : | |
| **concentration** | : | at °C |
| **Temperature effects** | : | |
| **Examine different pol.** | : | |
| **pKa** | : | at 25 °C |
| **Description** | : | |
| **Stable** | : | |
| **Deg. product** | : | |
| **Method** | : | |
| **Year** | : | 1991 |
| **GLP** | : | |
| **Test substance** | : | |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (4) not assignable |
| **27.01.2003** | | (12) |
| **Solubility in** | : | Water |
| **Value** | : | = 1.17 g/l at 20 °C |

| | | |
|---|---|---|
| **pH value** | : | |
| **concentration** | : | at °C |
| **Temperature effects** | : | |
| **Examine different pol.** | : | |
| **pKa** | : | at 25 °C |
| **Description** | : | |
| **Stable** | : | |
| **Deg. product** | : | |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Test not made in GLP, on the substance of highest purity. |
| **27.01.2003** | | (11) |

| | | |
|---|---|---|
| **Solubility in** | : | Water |
| **Value** | : | = 1.212 g/l at 25 °C |
| **pH value** | : | |
| **concentration** | : | at °C |
| **Temperature effects** | : | |
| **Examine different pol.** | : | |
| **pKa** | : | at 25 °C |
| **Description** | : | |
| **Stable** | : | |
| **Deg. product** | : | |
| **Method** | : | |
| **Year** | : | 1926 |
| **GLP** | : | no |
| **Test substance** | : | no data |
| **Result** | : | Other value : 2.423 mg/l at 40 °C. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Cited in BUA report 28, 1988. |
| **27.01.2003** | | (13) |

### 2.6.2 SURFACE TENSION

## 2.7 FLASH POINT

| | | |
|---|---|---|
| **Value** | : | = 167 °C |
| **Type** | : | other |
| **Method** | : | other: no data |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test substance** | : | Substance from Hoechst production |
| **Reliability** | : | (2) valid with restrictions |
| **24.04.2001** | | (14) |

## 2.8 AUTO FLAMMABILITY

| | | |
|---|---|---|
| **Value** | : | 519 °C at |

| | | |
|---|---|---|
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | ignition temperature |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test substance** | : | Substance from Hoechst production |
| **Reliability** | : | (4) not assignable |
| **23.04.2001** | | (15) |
| **Value** | : | ca. 521 °C at |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | ignition temperature (DIN 51794) |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test substance** | : | Substance from Bayer production |
| **Reliability** | : | (4) not assignable |
| **23.04.2001** | | (16) |
| **Value** | : | = 521 °C at |
| **Method** | : | |
| **Year** | : | 1987 |
| **GLP** | : | |
| **Test substance** | : | |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Data from Handbook |
| **23.04.2001** | | (17) |

## 2.9 FLAMMABILITY

## 2.10 EXPLOSIVE PROPERTIES

| | | |
|---|---|---|
| **Result** | : | not explosive |
| **Method** | : | Directive 84/449/EEC, A.14 "Explosive properties" |
| **Year** | : | |
| **GLP** | : | no |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Result** | : | Julius Peter test was negative.<br>Koënen test was negative for particle > 1 mm diameter. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Current guidelines followed on known substance, but not in GLP. |
| **24.04.2001** | | (11) |
| **Result** | : | other |
| **Method** | : | other |
| **Year** | : | |
| **GLP** | : | no |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Method** | : | Dust explosion assay in a sphere. |
| **Result** | : | Ignition Minimal Concentration : 30 g/m3<br>Ignition Minimal Energy : 5 mJ<br>Maximal explosion pressure : 8.6 bars<br>KST : 271 bars.m/sec |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Conclusion** | : | These results lead to conclude that when substance dusts are produced, |

they are sensitive to energy sources and present an explosion hazard when dispersed in the air.
The explosion effects are severe (classification ST2).

**Reliability** : (2) valid with restrictions
**23.04.2001** (18)

**2.11 OXIDIZING PROPERTIES**

**2.12 DISSOCIATION CONSTANT**

**2.13 VISCOSITY**

**2.14 ADDITIONAL REMARKS**

### 3.1.1 PHOTODEGRADATION

| | | |
|---|---|---|
| **Type** | : | air |
| **Light source** | : | |
| **Light spectrum** | : | nm |
| **Relative intensity** | : | based on intensity of sunlight |
| **INDIRECT PHOTOLYSIS** | | |
| **Sensitizer** | : | OH |
| **Conc. of sensitizer** | : | 500000 molecule/cm³ |
| **Rate constant** | : | = .0000000000343 cm³/(molecule*sec) |
| **Degradation** | : | ca. 50 % after .5 day(s) |
| **Deg. product** | : | |
| **Method** | : | other (calculated): Atkinson |
| **Year** | : | 1987 |
| **GLP** | : | |
| **Test substance** | : | |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Acalculation method has been used. |

**24.04.2001** (19)

### 3.1.2 STABILITY IN WATER

### 3.1.3 STABILITY IN SOIL

### 3.2.1 MONITORING DATA

| | | |
|---|---|---|
| **Type of measurement** | : | background concentration |
| **Media** | : | surface water |
| **Concentration** | : | = 1 µg/l |
| **Method** | : | GC-MS analysis |
| **Result** | : | The estimated half-life for ortho-nitroaniline was 2.3 days from Lobith to Gorinchem. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | On 16 July 1979 a sampling of Rhine water at Lobith (Netherland) and 24.5 hours later at Gorinchem (Netherland) was carried out for quantification of chemicals in the water by GC-MS analyses. |
| **Reliability** | : | (3) invalid<br>Few information were given concerning the test conditions and only two observations at one day time interval were performed. |

**24.04.2001** (20)

| | | |
|---|---|---|
| **Type of measurement** | : | background concentration |
| **Media** | : | surface water |
| **Concentration** | : | |
| **Method** | : | Gas chromatography and mass-spectrometry |
| **Result** | : | Ortho-nitroaniline has been identified in Wall river but no information concerning the concentrations were given. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Water was taken form Waal river at Brakel (Netherland). No information concerning sampling were given. |
| **Reliability** | : | (3) invalid<br>No concentrations has been specified. Moreover the test conditions were not well described. |

**24.04.2001** (21)

### 3.2.2 FIELD STUDIES

### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

| | | |
|---|---|---|
| **Type** | : | fugacity model level I |
| **Media** | : | |
| **Air** | : | .8 % (Fugacity Model Level I) |
| **Water** | : | 93.2 % (Fugacity Model Level I) |
| **Soil** | : | 5.8 % (Fugacity Model Level I) |
| **Biota** | : | % (Fugacity Model Level II/III) |
| **Soil** | : | % (Fugacity Model Level II/III) |
| **Method** | : | |
| **Year** | : | |
| **Method** | : | MacKay Fugacity model, level I, with Mp = 70°C; LogKow = 1.85; Vp = 0.368 Pa; water solubility = 1170 g/m3. |
| **Reliability** | : | (2) valid with restrictions<br>Calculation method |

**27.01.2003**

### 3.3.2 DISTRIBUTION

| | | |
|---|---|---|
| **Media** | : | water - air |
| **Method** | : | other (measurement): thermodynamic method for Henry law constant determination |
| **Year** | : | 1999 |
| **Method** | : | Method described in Brunner et al. (1990).<br>This method consist in the combination of a kinetic method based on the rate of loss of a substance from water by stripping with a gas and the static thermodynamic method which is the direct determination of the equilibrium concentrations in the two phases.<br>The pure substance was dissolved in demineralized water to a maximal concentration of 200 mg/L.<br>At 25°C, number of experimental run = 6. |
| **Result** | : | Experimentally determined dimensionless Henry's law constant at 25°C = 2.4 10-6 +/- 1.3 10-7 (SD) equivalent to 5.9 10-8 atm.m3/mol |
| **Reliability** | : | (2) valid with restrictions<br>Method well described, but no information concerning the test substance were provided. |

**11.02.2003** (22) (23)

### 3.4 MODE OF DEGRADATION IN ACTUAL USE

### 3.5 BIODEGRADATION

| | | |
|---|---|---|
| **Type** | : | aerobic |
| **Inoculum** | : | predominantly domestic sewage, non-adapted |
| **Concentration** | : | 100 mg/l related to Test substance<br>related to |
| **Contact time** | : | |
| **Degradation** | : | = 0 (±) % after 14 day(s) |
| **Result** | : | under test conditions no biodegradation observed |
| **Control substance** | : | other: aniline |
| **Kinetic** | : | %<br>% |

| | | |
|---|---|---|
| **Deg. product** | : | |
| **Method** | : | OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)" |
| **Year** | : | 1992 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | The inoculum was an activated sludge that was a mixture from 10 different sources in Japan (3 city sewage plants, 1 industry sewage plant, 3 river water samples, 1 lake water sample, and 2 bay seawater samples. This mixture was cultivated at 25 +-2 °C, with sythetic sewage as nutrient (made with glucos e, peptone and potasium phosphate). The activity of this inoculum was controled by testing it on a reference substance (aniline). The test was performed at 25 +- 1 °C, with the substance as sole source of carbon. The biodegradation percentage was calculated by ratio of BOD measured in a closed respirometer to Theoretical oxygen demand. |
| **Reliability** | : | (2) valid with restrictions<br>The test was performed according to OECD Guidelines and the data were validated by Japanese Competent Authorities. However the origin of the substance was not given. |

**01.08.2001** (24)

| | | |
|---|---|---|
| **Type** | : | aerobic |
| **Inoculum** | : | activated sludge, industrial, adapted |
| **Concentration** | : | 400 mg/l related to DOC (Dissolved Organic Carbon)<br>related to |
| **Contact time** | : | |
| **Degradation** | : | = 0 (±) % after 23 day(s) |
| **Result** | : | |
| **Deg. product** | : | |
| **Method** | : | OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test" |
| **Year** | : | 1990 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Remark** | : | No information were given concerning the kinetic of degradation of a reference substance. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Inoculum provided by Hoechst AG (1.1 g/l) |
| **Conclusion** | : | This inherent biodegradability test performed with industrial activated sludge from one of previous 2-nitroaniline producers, so adapted to the substance shows the non biodehradability of the substance. |
| **Reliability** | : | (2) valid with restrictions<br>No available data concerning the purity/supplier of the test substance, but test performed according to an OECD guideline, and with inoculum adapted to the substance. |

**25.04.2001** (25)

| | | |
|---|---|---|
| **Type** | : | aerobic |
| **Inoculum** | : | activated sludge, industrial, non-adapted |
| **Contact time** | : | |
| **Degradation** | : | ca. 25 (±) % after 25 day(s) |
| **Result** | : | under test conditions no biodegradation observed |
| **Kinetic of testsubst.** | : | 3 hour(s) 10 - 20 %<br>5 day(s) = 22 %<br>10 day(s) = 30 %<br>%<br>% |
| **Deg. product** | : | not measured |
| **Method** | : | OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test" |

| | | |
|---|---|---|
| **Year** | : | 1976 |
| **GLP** | : | no |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | Elimination of chemical oxygen demand: 25 % after 25 days, mainly by adsorption, as 10-20 % were already eliminated after 3 hours. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>Test performed according to a known method, but at a date when the OECD Guideline was not existing. However the test was probably performed with a substance of maximal purity, as produced by a Company whih declare a purity > 99 %. |

**22.06.2001** (26)

| | | |
|---|---|---|
| **Type** | : | Aerobic |
| **Inoculum** | : | activated sludge |
| **Contact time** | : | |
| **Degradation** | : | (±) % after |
| **Result** | : | under test conditions no biodegradation observed |
| **Deg. product** | : | |
| **Method** | : | other |
| **Year** | : | 1974 |
| **GLP** | : | No |
| **Test substance** | : | no data |
| **Remark** | : | There was no apparent biodegradation of the test substance under the cited test conditions (addition of 5 mg/l o-nitroanilin in sewage during a 10 day incubation period and then addition of 45 mg/l o-nitroaniline). No use of a reference substance. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Use of frozen sewage taken at one time to minimize variability in term of BOD and organisms present compared to samples taken at different times. The electrolytic respirometer was used to measure oxygen uptake rates. |
| **Reliability** | : | (3) invalid<br>No precise information were available concerning the test protocol and no information were available related to the test substance. |

**22.06.2001** (27)

| | | |
|---|---|---|
| **Type** | : | aerobic |
| **Inoculum** | : | other bacteria: Soil micro-organisms |
| **Concentration** | : | 10 mg/l related to Test substance<br>related to |
| **Contact time** | : | 64 day(s) |
| **Degradation** | : | = 0 (±) % after 64 day(s) |
| **Result** | : | under test conditions no biodegradation observed |
| **Deg. product** | : | no |
| **Method** | : | other: |
| **Year** | : | 1966 |
| **GLP** | : | no |
| **Test substance** | : | no data |
| **Result** | : | No ring cleavage after 64 day, so no primary biodegradation.<br>It was demonstrated that the chemical at the concentration employed was not toxic to the microflora. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | 40 ml of an appropriate medium were inoculated with 1 ml of a 1% suspension of Niagara sil loam (test substance is the sole source of carbon for microorganisms). Results were obtained at intervals of 3 to 6 hours and at 1, 2, 4, 8, 16,32 and 64 days after inoculation. The absorbancy of the supernatant was read at the selected wavelength against the supernatant from the reaction vessel containing a soil medium mixture free of the chemical but incubated in an identical fashion, so the primary biodegradation was appraised. |

**Conclusion** : These result shows no primary degradation, so no under-products are produced.
**Reliability** : (2) valid with restrictions
Protocol was described, but no information concerning the test substance were provided.
**22.06.2001** (28)

**Type** : Aerobic
**Inoculum** : activated sludge, adapted
**Concentration** : 200 mg/l related to COD (Chemical Oxygen Demand)
related to
**Contact time** :
**Degradation** : 0 (±) % after 20 day(s)
**Result** : under test conditions no biodegradation observed
**Deg. product** :
**Method** : other: Batch-Test, Evaluation of the degradation and of the degradation rate, based on the reduction of the chemical oxygen demand and TOC
**Year** : 1976
**GLP** : No
**Test substance** : no data
**Remark** : The biodegradability on the basis of the chemical oxygen demand is 0 %. No use of a reference substance.
**Source** : Rhodia Recherches Saint Fons
**Test condition** : The test substance is dissolved in a synthetic medium and thickened adapted activated sludge is added as inoculum. The test substance is the sole carbon source for the micro-organism of the inoculum.
**Reliability** : (3) invalid
The study is not reliable for biodegradability assesssment in the environment, as the test was not performed according standardised Guidelines and it was carried out with an adapted inoculum. Moreover, there were no information related to the test substance.
**22.06.2001** (29)

**Type** : Aerobic
**Inoculum** : Pseudomonas sp. (Bacteria)
**Contact time** : 20 day(s)
**Degradation** : = 1.9 (±) % after 20 day(s)
**Result** : under test conditions no biodegradation observed
**Kinetic of testsubst.** : %
10 day(s) = 1 %
20 day(s) = .9 %
%
%
**Deg. product** :
**Method** : other: Evaluation of degradation with a radiolabelled substrate, [14C]-Methode (Tracer analysis), evaluation of the release of CO2
**Year** : 1983
**GLP** : no data
**Test substance** :
**Result** : No degradation after 16 days.
**Source** : Rhodia Recherches Saint Fons
**Test condition** : Pseudomonas sp strain P6 (soil bacteries) was added to the test medium. O-nitroaniline was the sole source of carbon.
**Test substance** : purity > 98%
**Reliability** : (3) invalid
The study is not reliable for assessment of biodegradability in the environment as the inoculum is a pure bacterial strain.
**25.04.2001** (30)

**Type** : Aerobic

| | | |
|---|---|---|
| **Inoculum** | : | activated sludge, adapted |
| **Concentration** | : | 500 mg/l related to Test substance<br>related to |
| **Contact time** | : | 8 day(s) |
| **Degradation** | : | (±) % after |
| **Result** | : | under test conditions no biodegradation observed |
| **Deg. product** | : | |
| **Method** | : | other: Respirometric Test |
| **Year** | : | 1960 |
| **GLP** | : | No |
| **Test substance** | : | no data |
| **Remark** | : | Elimination of ca. 25 % probably by adsorption.<br>No more information were given concerning the kinetic. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | The conventional Warburg method was used. Incubation was at 20°C for 120 to 192 hours. Each flask was set up to contain 2,500 mg/l activated sludge solids and 500 mg/l test compound in a total volume of 20 ml. Oxidation was recorded as mg O2 uptake by liter of the mixture in the flask. A control flask for measurement of endogenous respiration was included in each run. |
| **Test substance** | : | Test substance was of analytical grade |
| **Reliability** | : | (3) invalid<br>The study is not reliable for biodegradability assessment in the environment as the inoculum was adapted, and the protocol was far from the OECD guidelines. |

**22.06.2001** (31)

| | | |
|---|---|---|
| **Type** | : | Aerobic |
| **Inoculum** | : | activated sludge, non-adapted |
| **Concentration** | : | 100 mg/l related to Test substance<br>related to |
| **Contact time** | : | 14 day(s) |
| **Degradation** | : | 0 (±) % after 10 day(s) |
| **Result** | : | under test conditions no biodegradation observed |
| **Deg. product** | : | |
| **Method** | : | other: Respirometric Test |
| **Year** | : | 1986 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Remark** | : | No biological degradation after 14 days.<br>No reference substance used. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Concentration of activated sludge: 30 mg/l<br>Culture medium: JIS inorganic mediums, 1 ml/300 ml<br>Temperature: 20 +/- 1 °C<br>pH of solution: 7 +/- 1<br>The measurement of BOD curves and the concentrations of DOC were repeated two or three times, and the reproductibility was confirmed. |
| **Reliability** | : | (2) valid with restrictions<br>as the test substance is not described and as the method is described in another publication. |

**25.04.2001** (32)

| | | |
|---|---|---|
| **Type** | : | Aerobic |
| **Inoculum** | : | domestic sewage, adapted |
| **Concentration** | : | 100 mg/l related to DOC (Dissolved Organic Carbon)<br>related to |
| **Contact time** | : | 14 day(s) |
| **Degradation** | : | (±) % after |
| **Result** | : | under test conditions no biodegradation observed |

| | | |
|---|---|---|
| **Deg. product** | : | |
| **Method** | : | other: measurement of ATP content |
| **Year** | : | 2000 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Remark** | : | ATP content in solution was measured. Results are expressed in terms of "Peak time" and "Peak time index" (curve peak height of organic substances test/curve peak height of endogenous test). The first parameter caracterizes the degradation rate of the test substance and the second one reflects the inhibition of test substances to microorganisms. The AI (Aggregate index of biodegradation) defined as the ratio "Peak height index/peak time *100" is < 50 for o-nitroanilin, indicating that the substance is poorly biodegradable.<br>No more information were given concerning the kinetic. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Amount of inoculum in the biological medium: 500 mg/l (as MLSS); Temperature of water bath: 20 +/- 1 °C; Initial pH value: 7.5 +/- 0.1; Simultaneously a blank (no test organic substance, no inoculum sludge) and an endogenous test (no test organic substance) were taken. |
| **Reliability** | : | (3) invalid<br>The ATP content measurement is not consistent with current OECD Guidelines. Moreover, the inoculum was adapted and the study cannot be relied on for assessment of biodegradability in the environment. |

**25.04.2001** (33)

| | | |
|---|---|---|
| **Type** | : | Anaerobic |
| **Inoculum** | : | other bacteria: Veiflonella alkalescens (cell-free extract) |
| **Deg. product** | : | |
| **Method** | : | other |
| **Year** | : | 1976 |
| **GLP** | : | No |
| **Test substance** | : | other TS |
| **Remark** | : | The rate of hydrogene consumption by the cell free extract on orthonitroaniline compound was 23 nmol/min * mg protein.<br>No more information were given concerning the kinetic. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test substance** | : | Test substance was from Eastman Kodak Co. |
| **Reliability** | : | (3) invalid<br>This study is not reliable for assessment of biodegradability in the environment as the method is far from standardized Guidelines (cell-fre extract were used as reagent, it was not an inoculum as such.) |

**22.06.2001** (34)

| | | |
|---|---|---|
| **Type** | : | |
| **Inoculum** | : | activated sludge, domestic |
| **Concentration** | : | 10 mg/l related to Test substance<br>related to |
| **Contact time** | : | 60 day(s) |
| **Degradation** | : | (±) % after |
| **Result** | : | other |
| **Deg. product** | : | yes |
| **Method** | : | other |
| **Year** | : | 1983 |
| **GLP** | : | no data |
| **Test substance** | : | other TS |
| **Remark** | : | Degradation of o-Nitroaniline to 2-Nitroacetanilide and 2-methylbenzimidazole. |
| **Result** | : | Under aerobic conditions, a significant amount of the absorbancy of orthonitroaniline remained after 53 days.<br>Under anaerobic conditions, the absorbancy of orthonitroaniline was |

appreciably reduced after 28 days.
No more information were given concerning the kinetic.

**Source** : Rhodia Recherches Saint Fons

**Test condition** : Sewage were incubated under aerobic or anaerobic conditions. Incubation in the dark at 29 °C, pH 7.3 to 8.5. Fresh sewage (5% vol/vol) was added every 7 days. Samples were taken at 0, 2 and 7 days and at weekly intervals thereafter. Analysis were performed on the supernatant. Disappearence of the test compounds, which was measured with a double beam spectrophotometer, was determined by dividing the area of the UV peak in nonsterile samples by the area in steril controls analyzed at the same time.

**Test substance** : Test substance was obtained from Eastman Kodak Co. and was of the highest purity available and was not purified further.

**Reliability** : (2) valid with restrictions
The protocol does not entirely fulfil the requirement of standardized method, but is well described.

**25.04.2001** (35)

### 3.6 BOD5, COD OR BOD5/COD RATIO

### 3.7 BIOACCUMULATION

**Species** : Brachydanio rerio (Fish, fresh water)
**Exposure period** : 23 hour(s) at 26 °C
**Concentration** : .2 µmol/l
**BCF** : 8.1
**Elimination** : Yes
**Method** : other
**Year** : 1991
**GLP** : no data
**Test substance** :

**Result** : The equilibrium of concentrations in fish was reached after 3 hours exposure.
The absence of C14 in the air extracted shows that no volatile metabolites are formed.
The concentration in fish at the end of the elimination period was 3.6 % of the steady state value.
Th radiioactivity in fish after 48 hour elimination was 3.6% of equilibrium concentration.

**Source** : Rhodia Recherches Saint Fons

**Test condition** : Exposure was performed under static conditions in closed basin (5 l of carbon filtered tap water, pH of 8,1 +/- 0,1; temperature = 26 +/- 1 °C) with 60 fishes of both sexes weighing 150 - 450 mg.
Fishes were from West Aquarium , Bad Lauterberg (FRG).
Concentrations were measured n the fish evry hour during the first 4 then every 3rd-10th hour.
Elimination was measured during 48 hours.

**Test substance** : C14 labelled compound were obtained from Sigma.
Radiochemical purity was tested with HPLC or TLC prior to the experiments. If required, purification was carried out by HPLC.

**Reliability** : (1) valid without restriction
The test was not performed according to standardized Guidelines but was consistent with them and very adequately conducted.

**03.07.2001** (36)

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 42 day(s) at 25 °C

| | | |
|---|---|---|
| Concentration | : | .5 mg/l |
| BCF | : | 2.1 - 4.9 |
| Elimination | : | Yes |
| Method | : | OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish" |
| Year | : | 1992 |
| GLP | : | no data |
| Test substance | : | no data |
| Result | : | At a concentration of 0.05 mg/l, the BCF was <10. |
| Source | : | Rhodia Recherches Saint Fons |
| Test condition | : | Test fish:<br>Cyprinus Carpio from Sugishima fish farm (Kumamoto, Japan).<br>Fish were reared in an acclimation tank in a flow through system at temperature of 25 +/- 2 °C for about 28 days.<br>During the period, abnormal fishes were removed. Then the fishes were exposed to the test substance in a flow through system for about one month. At the initiation of exposure the weight was about 30 g, the length was about 10 cm and the lipid content was 2-6 %.<br>Test conditions:<br>- flow through system<br>- glass tank of 100 l<br>- flow rate of test water: 200-800 ml/mn<br>- temperature of test water: 25 +/- 2 °C<br>- concentration of the dissolved oxygen in the test tank: 6-8 mg/l<br>- no information on oxygen content or pH during testing<br>- number of fishes at the initiation of exposure: 15-20 fishes/level<br>- duration of exposure 6 weeks<br>- preparation of a stock solution of test substance 100 times more concentrated than that in the aquarium<br>- the test substance concentrations were measured<br>- the test water was analysed twice a week and some test fishes were analysed every two weeks |
| Reliability | : | (1) valid without restriction<br>The test was performed according to OECD Guidelines and the data were validated by Japanese Competent Authorities. |

20.04.2001 (37)

**3.8 ADDITIONAL REMARKS**

## 4.1 ACUTE/PROLONGED TOXICITY TO FISH

| | | |
|---|---|---|
| **Type** | : | other: no information |
| **Species** | : | Carassius auratus (Fish, fresh water) |
| **Exposure period** | : | 48 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | = 11.5 |
| **Limit test** | : | |
| **Analytical monitoring** | : | no data |
| **Method** | : | other |
| **Year** | : | 1997 |
| **GLP** | : | No |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | No data.<br>The toxicity values and confidence intervals were determined by probit analysis. |
| **Reliability** | : | (3) invalid<br>No information were given concerning the test conditions and the substance tested. |

**26.04.2001** (38)

| | | |
|---|---|---|
| **Type** | : | Semistatic |
| **Species** | : | Brachydanio rerio (Fish, fresh water) |
| **Exposure period** | : | 96 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | = 19.5 |
| **Limit test** | : | |
| **Analytical monitoring** | : | Yes |
| **Method** | : | other: OECD, 1984, Guidelines for Testing of Chemicals. OECD, Paris |
| **Year** | : | 1991 |
| **GLP** | : | no data |
| **Test substance** | : | other TS |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Test Fish:<br>Zebrafish obtained from West Aquarium, Bad Lauterberg (FRG). The age of the fish was about 3 months and the weight ranged between 200 and 350 mg. Both sexes were used. Fishes were not fed 24 h prior to testing and during the 96 h exposure period. A 12h light-12h dark photoperiod was used.<br><br>Test conditions:<br>The test water was charcoal-filtered, aerated tap-water, which was mixed with a stock solution of the chemical in distilled water. The pH was 8,6 +/- 0,3; the dissolved oxygen was 85 +/- 15 % and the temperature was 26,5 +/- 1°C.<br>The concentrations were measured photometrically once a day and the test solutions were renewed if required.<br><br>Results:<br>LC50 values were calculated using a computer program based on the method of Litchfield and Wilcoxon (1949). |
| **Test substance** | : | o-nitroaniline was purchased from Merck-Schuchard (Hohenbrunn, FRG) |
| **Reliability** | : | (1) valid without restriction<br>The test was performed according OECD Guidelines with analytical control. Results described in Hoechst (1991) did not show less toxicity of the commercialised substance : it can be considered that the substance tested does not contain impurities showing toxicity. |

**23.05.2001** (39)

| | | |
|---|---|---|
| **Type** | : | Semistatic |
| **Species** | : | Carassius auratus (Fish, fresh water) |
| **Exposure period** | : | 48 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | = 1.66 |
| **Limit test** | : | |
| **Analytical monitoring** | : | no data |
| **Method** | : | OECD Guide-line 203 "Fish, Acute Toxicity Test" |
| **Year** | : | 1996 |
| **GLP** | : | No |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Test fish:<br>Carassius auratus were purchased from a commercial source (hatched about 35 days, Nanjing, China) and kept 10 days in the experimental water for acclimation before the test. Each fish was approximately 3.5 g weight and 4.0 cm length.<br>Fishes were not fed during the exposure to chemical.<br><br>Test conditions:<br>- semistatic test (water renewal at each 12 hr)<br>- 4 fishes in each 6-L glass beaker containing 4 L experimental solutions<br>- 16 hr light / 8 hr darkness as photoperiod<br>- conditions of the experimental water: temperature: 20 +/- 1 °C, dissolved oxygen: 8.2 +/- 0.5 mg/l; pH 7.5 +/- 0.3; hardness (as CaCO3) 110 +/- 10 mg/l<br>- test substance was purchased from Shangai Chemical Agent Co. (Shangai, China) and had a purity of > 95%<br>- 4 to 6 concentrations were tested with two replicates at each concentration<br><br>Results:<br>LC50 values were determined after the probit transformation of the lethal percentage of the fish. |
| **Test substance** | : | purity > 95% |
| **Reliability** | : | (3) invalid<br>A lack of data on identification and quantification of impurities is a major factor of invalidation of a study. However, if such a study result is consistent with most of validated results, or if doubtful results are within the same range of magnitude, they can validate each other, because the probability to have got the exact value is higher. By the contrary, if one result is within a different range from other values, the lack of data on impurities becomes very important, because there it is likely that the abnormal toxicity value is due to an unknown impurity. When the fish LC50 data set of 2-nitroaniline is analysed, it can be observed that one of the four validated value is one order of magnitude below this of the other values :<br>- LC50 96h Brachydanio rerio = 19.5 mg/l (assigned Validity 1)<br>- LC50 48h Carassius auratus = 1.66 mg/l (assigned Validity 2)<br>- LC50 96h Cyprinus carpio = 16.2 mg/l (assigned Validity 2)<br>- LC50 96h Brachydanio rerio = 10-22 mg/l (assigned Validity 2)<br><br>Several reasons are leading to invalidate the Carassius auratus LC50 value :<br>a) it is one order of magnitude below this of the 3 other values, and particularly than the only Validity 1 value.<br>b) The purity announced in the publication is > 95 %, which lets the opportunity of occurrence of a toxic impurity<br>c) Carassius auratus is a Cyprinides, like the 3 other fish and its sensitivity |

is not supposed to be very different
d) In the same publication, a LC50 48h on 4-nitroaniline has been found to be 1.2 mg/l. However, the fish toxicity data found in the IUCLID file are :
- LC50 96h Pimephales promelas = 106 mg/l
- LC50 96h Brachydanio rerion = 89 mg/l
- LC50 48h Leuciscus idus = 35 mg/l
- LC50 96h Oryzias latipes = 84 mg/l
- LC50 48h Salmo gairdneri = 28-56 mg/l
As the substance is neither biodegradable nor adsorbable nor volatile, an underestimation of toxicity due to loss of substance is unlikely. Moreover, the IUCLID data set is rather consistent, and the value found in this publication is clearly out of this range. This confirms the hypothesis that the nitroaniline samples tested were containing some impurities more toxic that the substance itself.
The result is therefore considered as invalid.

**09.08.2001** (40)

| | | |
|---|---|---|
| **Type** | : | Semistatic |
| **Species** | : | Cyprinus carpio (Fish, fresh water) |
| **Exposure period** | : | 96 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | = 16.2 |
| **Limit test** | : | |
| **Analytical monitoring** | : | no data |
| **Method** | : | other |
| **Year** | : | 1996 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Test fish:<br>One year old carps (Cyprinus carpio) were provided by Changchun Aquatic Institute reared under the laboratory conditions for 2 weeks. The average weight was 23.8 +/- 6.4 g and the average length was 11.6 +/- 2.3 cm.<br><br>Test conditions:<br>- Dechlorinated tap water with 21.45 mg/l chlorine; temperature: 15-18 °C; content in dissolved oxygen: 6.35 mg/l (12.3 °C); pH: 7.0-7.5<br>- semi-static test with renewal of the water twice a day and 10 l each time<br>- 60 L aquaria containing 20 l of test water and 10 fishes<br>- Acetone was used as solvent (0.05 - 0.1 % v/v)<br>- 5 concentration gradients were established<br>- Controls: same number of fishes and equal amount of solvent |
| **Reliability** | : | (2) valid with restrictions<br>The results have to be taken with precaution, as no analytical control was performed and no substance purity data was given. However, as the 2-nitroaniline is neither biodegradable nor volatile, nor particularly adsorbable, and the LC50 value looks consistent with values obtained with those obtained in other taxa, this value can be considered as acceptable fr the hazard assessment. |

**22.06.2001** (41) (42)

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Brachydanio rerio (Fish, fresh water) |
| **Exposure period** | : | 96 hour(s) |
| **Unit** | : | mg/l |
| **LC0** | : | = 10 |
| **LC50** | : | 10 - 22 |
| **LC100** | : | = 50 |
| **Limit test** | : | |

| | | |
|---|---|---|
| **Analytical monitoring** | : | No |
| **Method** | : | OECD Guide-line 203 "Fish, Acute Toxicity Test" |
| **Year** | : | 1991 |
| **GLP** | : | Yes |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | The LC50 (48 h) ranged from 22 to 50 mg/l. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>The test was performed according OECD Guidelines but concentrations were not measured. |

**26.04.2001** (43)

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Cyprinus carpio (Fish, fresh water) |
| **Exposure period** | : | 22 hour(s) |
| **Unit** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | No |
| **Method** | : | other: no data |
| **Year** | : | 1963 |
| **GLP** | : | No |
| **Test substance** | : | no data |
| **Remark** | : | Publication not available |
| **Result** | : | At test Dose (163 - 189 mg/kg) no mortality was observed and the behaviour was normal. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Diet exposure |
| **Reliability** | : | (3) invalid<br>The results are not reliable as the test was not performed according standardized method : exposure period was only 22 hours, fish were exposed by diet. |

**22.06.2001** (44)

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Leuciscus idus (Fish, fresh water) |
| **Exposure period** | : | 48 hour(s) |
| **Unit** | : | mg/l |
| **LC0** | : | = 10 |
| **Limit test** | : | |
| **Analytical monitoring** | : | No |
| **Method** | : | other: DIN 38412 Part 15 |
| **Year** | : | 1976 |
| **GLP** | : | No |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid<br>The test was performed according to standardized Guidelines without GLP and the concentrations were not measured. Moreover, the test duration was only 48 hours, and a LCO only was driven from the test. |

**26.04.2001** (26)

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Oryzias latipes (Fish, fresh water) |
| **Exposure period** | : | 48 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | = 17 |
| **Limit test** | : | |
| **Analytical monitoring** | : | No |
| **Method** | : | other: Japanese Industrial Standard (JIS K 0102-1986-71) |

| | | |
|---|---|---|
| **Year** | : | 1992 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Result** | : | Results given as nominal concentrations (no concentration measurement). Neither observation nor mortality tables available. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Test fishes:<br>Oryzias Latipes from Nakashima fish farm (Kunamoto, Japan), fish size not described, loading 10 fish / 4 l. Fishe were reared in an acclimatization tank in a flow through system at temperature of 25 +/- 2°C for about 28 days.<br><br>Test conditions:<br>- static or semi-static test ( renewal of test water at every 8-16 hours)<br>- dilution water: underground water pumped up from the ground of Kurume Research laboratories. Quality of dilution water was in compliance with the ministerial ordinance of the Ministry of Health and Welfare (31/08/1978) and water quality criteria for fisheries (Shandonhozin Nihon Suisansigen Hogokyokai (03/1983).<br>- test solution: preparation not described<br>- no information on tested concentrations<br>- test tank: round glass vessel (4 l)<br>- 10 fish/concentration<br>- no information on oxygen content, pH during testing<br>- test temperature: 25 +/- 2 °C<br>- calculation of LC50 48h by Doudoroff or probit method. |
| **Reliability** | : | (3) invalid<br>Data approved by the Japanese Competent Authorities, but neither analytical control of substance concentrations nor substance purity were described. |

**26.04.2001** (37)

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Petromyzon marinus |
| **Exposure period** | : | 24 hour(s) |
| **Unit** | : | |
| **Limit test** | : | |
| **Analytical monitoring** | : | no data |
| **Method** | : | other: Laboratory statistic methode |
| **Year** | : | 1957 |
| **GLP** | : | No |
| **Test substance** | : | no data |
| **Remark** | : | No effect was observed at tested concentrations |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Larvae, 8 - 13 cm |
| **Reliability** | : | (4) not assignable<br>The report was not available. |

**22.06.2001** (45)

### 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

| | | |
|---|---|---|
| **Type** | : | |
| **Species** | : | Daphnia magna (Crustacea) |
| **Exposure period** | : | 48 hour(s) |
| **Unit** | : | mg/l |
| **EC0** | : | = 5.6 |
| **EC50** | : | 10 - 18 |
| **EC100** | : | = 18 |

| | | |
|---|---|---|
| **Analytical monitoring** | : | No |
| **Method** | : | OECD Guide-line 202 |
| **Year** | : | 1991 |
| **GLP** | : | Yes |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | EC0 (24 h) = 5.6 mg/l<br>EC50 (24 h) = 11.8 - 15.2 mg/l<br>EC100 (24 h) = 32 mg/l |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>The test was performed according OECD Guidelines but no information were given concerning the type (static, semi-static or dynamic) and the concentrations were not measured. |

**26.04.2001** (46)

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | Daphnia magna (Crustacea) |
| **Exposure period** | : | 24 hour(s) |
| **Unit** | : | mg/l |
| **EC50** | : | = 8.3 |
| **Analytical monitoring** | : | No |
| **Method** | : | other |
| **Year** | : | 2000 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Result** | : | IC50 = 8.3 mg/l at pH 7.8 +/- 0.1<br>IC50 = 7.1 mg/l at pH 9.0<br>IC50 = 10 mg/l at pH 6.0 |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Test organisms:<br>Daphnia magna were cultured parthenogenetically in an environmental chamber at 22 +/- 2 °C, with a photoperiod of 14 h daylight/10 darkness. They were fed with a diet of green algae and 6-24 h old Daphnia magna were used for the test. They were not fed during experimentation.<br><br>Test conditions:<br>- Static method for 24 h<br>- 10 Daphnia magna in 25 ml of test water<br>- The test substance was diluted with reconstituted hard water<br>- No information were given concerning the stock solution preparation, the test temperature, the water chemistry and the lighting<br>- The substance was tested at 3 different pH (6.0 +/- 0.1; 7.8 +/- 0.1 and 9.0 +/- 0.1). The pH values were determined at the beginning and at the end of each test.<br>- The substance was tested at each pH at six different concentrations (no more information).<br>- Dissolved oxygen concentration was determined using iodometric titration (no more information).<br><br>Results:<br>The numbers of immobilized daphnies were determined after 24 h (3 determinations were performed). The IC50 at 24 h were calculated from the dose-response relationships using the MINITAB software. The results were considered valid if dissolved oxygen measured at the end of the test was at least 60 % of saturation and if the percentage of immobilisation observed for the controls was zero. |
| **Test substance** | : | The test substance was purchased as analytical pure. |
| **Reliability** | : | (2) valid with restrictions<br>The results have to be taken with precaution, as no analytical control was |

performed. Moreover few information were given concerning the test conditions, and there were no replicate per concentration. However, as the 2-nitroaniline is neither biodegradable nor volatile, nor particularly adsorbable, and the EC50 value looks consistent with values obtained with those obtained in other taxa, this value can be considered as acceptable fr the hazard assessment.

**06.08.2001** (47)

| | | |
|---|---|---|
| **Type** | : | |
| **Species** | : | Daphnia magna (Crustacea) |
| **Exposure period** | : | 48 hour(s) |
| **Unit** | : | mg/l |
| **EC50** | : | = 4.9 |
| **Analytical monitoring** | : | no data |
| **Method** | : | other: no information |
| **Year** | : | 1997 |
| **GLP** | : | no data |
| **Test substance** | : | other TS |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | No information<br><br>Results:<br>The EC50 and confidence i ntervals were determined by probit analysis. |
| **Test substance** | : | purity > 98 % |
| **Reliability** | : | (3) invalid<br>The results are not reliable as no information were given concerning the test conditions, and no analytical monitoring was made. |

**26.04.2001** (38)

| | | |
|---|---|---|
| **Type** | : | Static |
| **Species** | : | other: Daphnia carinata |
| **Exposure period** | : | 48 hour(s) |
| **Unit** | : | mg/l |
| **EC50** | : | = 10.5 |
| **Analytical monitoring** | : | no data |
| **Method** | : | other |
| **Year** | : | 1997 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Tests organisms:<br>Daphnia carinata was cultured parthenogenetically in an environmental chamber at 22 +/- 1 °C, with a photoperiod of 14 hours daylight/10 hours darkness. They were fed a diet of green algae and 2-24 h old Daphnia carinata were used for the test. The Daphnia were not fed during the test.<br><br>Test conditions:<br>- Static method for 48 h<br>- 10 Daphnia carinata in 25 ml of test water<br>- A total of 60 Daphnia carinata was used<br>- Stock solutions of chemical were prepared in acetone<br>- No more information concerning the test water, temperature, pH... were given.<br><br>Results:<br>The number of immobilisation were determined regularly.<br>The results were considered valid if dissolved oxygen measured at the end of the test was at least equal to 60 % of saturation, and if the percentage of immobilization observed for the controls was zero. |

| | | |
|---|---|---|
| **Test substance** | : | The test substance was purchased from commercial source and was not repurified before testing. |
| **Reliability** | : | (2) valid with restrictions<br>The results have to be taken with precaution, as no analytical control was performed and no substance purity data was given. Moreover few informationwere given concerning the test conditions, probably only 10 organisms were tested per concentration. However, as the 2-nitroaniline is neither biodegradable nor volatile, nor particularly adsorbable, and the EC50 value looks consistent with values obtained with those obtained in other taxa, this value can be considered as acceptable fr the hazard assessment. |

**22.06.2001** (42)

### 4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

| | | |
|---|---|---|
| **Species** | : | Chlorella vulgaris (Algae) |
| **Endpoint** | : | Biomass |
| **Exposure period** | : | 6 hour(s) |
| **Unit** | : | mg/l |
| **EC50** | : | = 91.26 |
| **Limit test** | : | |
| **Analytical monitoring** | : | No |
| **Method** | : | other: Standardised growth test. BOHM et al., (1972): Selection method of biochemical active substances. DD Nr 94234/C 12K 1/00 |
| **Year** | : | 1986 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | test organisms:<br>Chlorella vulgaris<br><br>test conditions:<br>- test medium: prepared according Bohm 1973 (Wiss. Hefte d. Pad. Inst. Koten 2, 217-220)<br>- Algae concentration: CA 7.5 x 10E6 spore/ml<br>- Temperature: 36.5 °C |
| **Test substance** | : | Analytical control of the purity. |
| **Reliability** | : | (3) invalid<br>The results are not reliable as the test was not performed according to a standardized Guidelines. Moreover, the test duration was only 6 hours. |

**23.05.2001** (48)

| | | |
|---|---|---|
| **Species** | : | other algae: Scenedesmus obliquus |
| **Endpoint** | : | growth rate |
| **Exposure period** | : | 96 hour(s) |
| **Unit** | : | mg/l |
| **EC50** | : | = 64.6 |
| **Limit test** | : | |
| **Analytical monitoring** | : | no data |
| **Method** | : | other |
| **Year** | : | 1997 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Test organisms:<br>Green algae (Scenedesmus obliquus) were cultured in the medium at 24 +/- 1°C under cool white fluorescent light of 4000 Lux +/- 10 % with a light cycle |

of 12 hours on 24 hours.

Test conditions:
- The test algae were cultured in 50 ml solution containing five different concentrations of test compound in 100 ml sterile closed flasks.
- The initial algae density was 10E4 cell/ml.
- Triplicate exposure samples of test solution and controls were used in the experiment.
- The growth of algae was monitored by measuring the cell density after 0, 24, 48, 72 and 96 hours and the optical density was determined at 96 hours at 650 nm.

Results:
The 96h-EC50 for growth inhibition was extrapolated from the empirical logarithmic curves with the percentage of growth inhibition in function of concentrations.

**Reliability** : (2) valid with restrictions
The results have to be taken with precaution, as no analytical control was performed and no substance purity data was given. However, as the 2-nitroaniline is neither biodegradable nor volatile, nor particularly adsorbable, and the EC50 value looks consistent with values obtained with those obtained in other taxa, this value can be considered as acceptable fr the hazard assessment.

**22.06.2001** (42)

### 4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

| | | |
|---|---|---|
| **Type** | : | aquatic |
| **Species** | : | activated sludge, domestic |
| **Exposure period** | : | 24 hour(s) |
| **Unit** | : | mg/l |
| **LOEC** | : | = 150 |
| **Analytical monitoring** | : | no |
| **Method** | : | ETAD Fermentation tube method "Determination of damage to effluent bacteria by the Fermentation Tube Method" |
| **Year** | : | 1976 |
| **GLP** | : | no |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | LOEC: Lowest Effect Concentration Level |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (4) not assignable<br>Report not available. The exact test conditions could not be checked. |

**25.06.2001** (26)

| | | |
|---|---|---|
| **Type** | : | aquatic |
| **Species** | : | Photobacterium phosphoreum (Bacteria) |
| **Exposure period** | : | 15 minute(s) |
| **Unit** | : | mg/l |
| **EC50** | : | = 26.9 |
| **Analytical monitoring** | : | no data |
| **Method** | : | other: Alsop G.M., Waggy G.T., Conway R.A. (1980): Bacterial growth inhibition test J. WPCF 52: 2452 |
| **Year** | : | 1997 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | The test was conducted using the Microtox toxicity analyzer (DXY-2, made by |

the Institute of Soil science, Academia Sinica, Nanjing, China).
The concentration values causing 50 % reduction of bioluminescence were performed at 20 °C according to the procedures described in the Instrumental Manual. All bioassays were carried out in duplicate or triplicate for statistical purpose.

**Reliability** : (2) valid with restrictions
The results are reliable with restrictionsbecause no data on substance purity were given.

**25.06.2001** (49)

**Type** : aquatic
**Species** : other bacteria: bacterial seed taken from the Shonhua river
**Exposure period** : 24 hour(s)
**Unit** : mg/l
**EC50** : = 34.7
**Analytical monitoring** : no data
**Method** : other: bacterial growth inibition test according to Alsop et al. (1980) J. WPCF 52:2452
**Year** : 1997
**GLP** : no data
**Test substance** : no data
**Source** : Rhodia Recherches Saint Fons
**Test condition** : Test organisms:
Bacterial seed take from the Songhua River.

Test conditions:
The mixtures (containing toxicant, buffering agent, nutrients, growth substrates and bacterial seed inocula) were incubated for 24 h at 22 +/- 2 °C. The turbidities were measured at 530 nm against a blank of an unseeded control.
Results:
The absorbance values of the toxicant-amended mixtures were calculated as a percentage of the control using the simple relationship as follow: Absorbance of test bottle/Absorbance of seed control x 100 = % of controls. The percentages of control values were plotted against the logarithm of the toxicant concentration and the IC50 (toxicant concentration reducing growth by 50%) was calculated from the plot. All bioassays were carried out in duplicate or triplicate for statistical purpose.

**Reliability** : (2) valid with restrictions
The results are reliable with restrictions as few information concerning the protocol were available. However the conditions were similar to a Pseudomonas putida test.

**25.06.2001** (49)

**Type** : other: anaerobic test
**Species** : other bacteria: acetoclastic methanogenic bacteria in n on adapted industrial sludge
**Exposure period** : 3 day(s)
**Unit** : mg/l
**EC50** : = 1.9
**Method** : other: methane production measurement by gas chromatography
**Year** : 1995
**GLP** : no data
**Test substance** : other TS
**Result** : IC (20%) = 7 µM
IC (50%) = 14 µM
IC (80%) = 70 µM
**Source** : Rhodia Recherches Saint Fons
**Test condition** : Inoculum:
Elutriated methanogenic granular sludge from the full scale upward-flow

anaerobic sludge blanket (UASB) reactor of Shell Nederland Chemie was used as inoculum.

Test conditions:
Sludge (2 g/l) was transferred to vials containing 25 ml of the basal medium and acetate (2.5 g COD/l). The desired amount of toxicant was added to duplicate vials. Triplicate substrate controls were based on assays where no toxicant was added. After 3 days of exposure to the toxicant (incubation temperature 30 +/- 2 °C), the acetate concentration was replenished to 1g COD/l to assess the specific methanogenic activity; the assay bottle were reincubated 1 h prior to the determination of the methane production rate. The methane content was determined hourly during 6 to 8 h incubation period. Unacclimated cultures were used to minimize the biotransformation of the toxic organic chemical during the test.

**Test substance** : highest purity available on the market and not purified further
**Reliability** : (2) valid with restrictions
The test was not performed according to standardized Guidelines, but well conducted on a high purity substance (commercialised substance can be > 99.6 %)

**04.07.2001** (50)

**Type** :
**Species** : activated sludge
**Exposure period** : 3 hour(s)
**Unit** : mg/l
**EC50** : 405
**Analytical monitoring** : no data
**Method** : other: ISO 8192
**Year** :
**GLP** : no data
**Test substance** : as prescribed by 1.1 - 1.4
**Remark** : direct weighing
**Source** : Rhodia Recherches Saint Fons
**Reliability** : (4) not assignable
The report is not available and few information were given concerning the protocol.

**26.04.2001** (51)

**Type** :
**Species** : Tetrahymena pyriformis (Protozoa)
**Exposure period** : 40 hour(s)
**Unit** : mg/l
**EC50** : = 115
**Method** : other: Test performed according to the method of Schultz (1997) Toxicol. Methods, 7, 289-309
**Year** : 1999
**GLP** : no data
**Test substance** : no data
**Source** : Rhodia Recherches Saint Fons
**Test condition** : Test organisms:
T.pyriformis (strain GL-C)

Test conditions:
- Static test (40 hours)
- The protocol was described by Schultz (Toxicol. Methods 7, 289-309,1997)
- The test protocol allows for eight to nine cell cycles in controls.
- Tests were performed in triplicate. Each replicate consisted of six to eight different concentrations with duplicate flasks with each concentration.
- Two controls were used (the first one had no test material and was

inoculated; the second one had neither test material nor inocula). Only replicate with control absorbency values of >0.6 but <0.75 were used.
- The population density was quantitated spectrophotometrically at 540 nm

**Test substance** : purity > 95%
**Reliability** : (2) valid with restrictions
The results are reliable as the test is well conducted, but substance purity not well known (> 95 %).

**25.06.2001** (52)

**4.5.1 CHRONIC TOXICITY TO FISH**

**4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES**

**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 TOX. TO OTHER NON MAMM. TERR. SPECIES**

**Species** : other avian
**Endpoint** : mortality
**Exposure period** :
**Unit** : mg/kg bw
**Method** : other
**Year** : 1983
**GLP** : no data
**Test substance** : no data
**Result** : LD50 (Agelaius phoenicus) = 750 mg/kg
LD50 (Sturnus vulgaris) > 1000 mg/kg
LD50 (Coturnix coturnix) = 750 mg/kg
**Source** : Rhodia Recherches Saint Fons
**Test condition** : Test organisms:
Red-winged blackbird (Agelaius phoenicus)
European starling (Sturnus vulgaris)
Coturnix (C oturnix coturnix)
Wild-trapped birds were preconditioned to captivity for 2 to 6 weeks.

Test conditions:
Birds were dosed by gavage with solutions or suspensions of the test substance in propylene glycol according to method described by DeCino et al. (J. Wildl. manage. 30, 249, 1966), Schafer (Toxicol. Appl. Pharmacol. 21, 315, 1972) and Schafer et al. (J. Agric. Food Chem. 15, 287, 1967).

Results:
LD50 values were calculated by the method of Thompson and Weil (Biometrics, 8, 51, 1952) and Weil (Biometrics, 8, 249, 1952).

**Test substance** : The test substance was of technical or analytical grade.
**Reliability** : (3) invalid
The results are not reliable as the test was not performed according to standardized Guidelines.

**24.04.2001** (53)

**4.7 BIOLOGICAL EFFECTS MONITORING**

**4.8 BIOTRANSFORMATION AND KINETICS**

**4.9 ADDITIONAL REMARKS**

## 5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

### 5.1.1 ACUTE ORAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | = 1838 mg/kg bw |
| **Species** | : | rat |
| **Strain** | : | Wistar |
| **Sex** | : | female |
| **Number of animals** | : | 10 |
| **Vehicle** | : | no data |
| **Doses** | : | 800, 1250, 1600, 2000 and 3200 mg/kg |
| **Method** | : | other: method from the laboratory, 5 animals per dose |
| **Year** | : | 1973 |
| **GLP** | : | no |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | Before GLPs. In agreement with other data (1977) Vernot |
| **Result** | : | All dose levels were administered by oral route (gavage).<br>The rats weighed 80-110g (average 94g) at study initiation.<br>The rats were observed for 14 days after exposure.<br>0, 0, 2, 7 and 10 rats died before the end of the observation period, for the respectively doses 800, 1250, 1600, 2000 and 3200 mg/kg. All animals dying spontaneously were grossly necropsied, as well as all rats that survived to the end of the 14-day study.<br>Observations : animals died having cramps, after exposure.<br>Animals were in a narcotic state. Urine was coloured orange.<br>Necropsy revealed no macroscopic lesions.<br>The LD50 is 1838 ( 1673-2018) mg/kg bw. |
| **Source** | : | INERIS |
| **Reliability** | : | (2) valid with restrictions<br>This report was sent to French CA by CLARIANT, and was examined previously by BUA. Only female were used and the reprot was done before GLPs. |
| **Flag** | : | confidential, Risk Assessment, Critical study for SIDS endpoint |

**11.02.2003** (54)

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | = 3650 mg/kg bw |
| **Species** | : | rat |
| **Strain** | : | Sprague-Dawley |
| **Sex** | : | male |
| **Number of animals** | : | 6 |
| **Vehicle** | : | water |
| **Doses** | : | 10-1-0.1 mg/kg |
| **Method** | : | other: Smyth et al. (1962) as described in remark |
| **Year** | : | 1977 |
| **GLP** | : | no data |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | Before GLPs.<br>Compound solubilised or dispersed in water at doses of 10-1-0.1 mg/kg and more if needed.<br>After a first dose a week of observation is done before starting the next dose, observation is done for 14 days.<br>This is descirbed as a range-finding method, with statistical analysis(moving average technique).<br>This report is dealing with many other chemicals among which the other isomers of ortho-nitraniline. The comparative LD50 reported are: |

ortho-NAniline: 3560 (2590-4910)mg/kg
meta-NAniline: 540 (360-790)mg/kg
para-NAniline: 3250 (1980-5700) mg/kg

**Source** : Rhodia Recherches Saint Fons
**Reliability** : (2) valid with restrictions
the exact method is described in a previous paper (Smyth,1962), no GLP due to the year of realisation.Normally rats are Sprague Dawley, but can be another strain, and weighing 90-120 g.
**Flag** : non confidential, Risk Assessment
**25.06.2002** (55) (56)

**Type** : LD50
**Value** : = 1600 mg/kg bw
**Species** : rat
**Strain** : no data
**Sex** : no data
**Number of animals** : 10
**Vehicle** : no data
**Doses** :
**Method** : other: Behrens and Sclosser
**Year** : 1966
**GLP** : no
**Test substance** : no data
**Remark** : Study performed before the GLPs exist, only a table to interpret, Russian.
Comparison between the 3 isomers
The 3 isomers were compared in rat, mouse and guinea-pig:

| | rat | mouse | guinea pig LD50's |
|---|---|---|---|
| ortho | 1600 | 1246 | 2350 |
| meta | 700 | 531 | 450 |
| para | 1500 | 1414 | 450 |

**Source** : Rhodia Recherches Saint Fons
**Reliability** : (3) invalid
No data concerning test substance, method, No of animals, no GLP due to the year of realisation.
Publication in Russian, only data tables are readable.
**Flag** : non confidential
**07.08.2001** (57)

**Type** : LD50
**Value** : = 535 mg/kg bw
**Species** : rat
**Strain** : no data
**Sex** : no data
**Number of animals** :
**Vehicle** : no data
**Doses** :
**Method** : other
**Year** : 1985
**GLP** : no data
**Test substance** : no data
**Remark** : This is an error of first IUCLID data set and is the value indicated for meta-nitroaniline in D.O.S.E.books (Dictionary of Substances and their effects).
**Source** : Rhodia Recherches Saint Fons
**Reliability** : (4) not assignable
The paper of SHAHIN does not mention any data on oral toxicity: it is a paper dealing with mutagenecity and will be examined in the related chapter.
**Flag** : non confidential
**27.08.2001** (58)

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | = 3520 mg/kg bw |
| **Species** | : | rat |
| **Strain** | : | no data |
| **Sex** | : | no data |
| **Number of animals** | : | |
| **Vehicle** | : | no data |
| **Doses** | : | |
| **Method** | : | other: no data |
| **Year** | : | 1974 |
| **GLP** | : | no |
| **Test substance** | : | no data |
| **Remark** | : | Before GLPs.<br>Comparison between the 3 isomers of Nitroaniline:<br>ortho: 3520 (2790-4430)<br>meta: 900 ( 700-1150)<br>para: 1410 (1020-1950) |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid<br>No data conceming the exact method, Number of animals, no GLP due to the year of realisation. Only a table , Russian.<br>Note only that the value indicated is not far from that reported by Vernot(1977). |
| **Flag** | : | non confidential |
| **06.08.2001** | | (59) |

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | = 1070 mg/kg bw |
| **Species** | : | mouse |
| **Strain** | : | no data |
| **Sex** | : | no data |
| **Number of animals** | : | |
| **Vehicle** | : | no data |
| **Doses** | : | |
| **Method** | : | other: no data |
| **Year** | : | 1981 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Remark** | : | comparison of the 3 isomers of nitroaniline with previous rat data and mouse now.<br>mouse LD50 in mg/kg:<br>ortho: 1070<br>meta: 420<br>para: 940 |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid<br>No data concerning test substance, method, No of animals, no GLP due to the year of realisation. Only table, Russian |
| **Flag** | : | non confidential |
| **06.08.2001** | | (60) |

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | = 1290 mg/kg bw |
| **Species** | : | mouse |
| **Strain** | : | no data |
| **Sex** | : | no data |
| **Number of animals** | : | |
| **Vehicle** | : | no data |
| **Doses** | : | |

| | | |
|---|---|---|
| **Method** | : | other: no data |
| **Year** | : | 1977 |
| **GLP** | : | no data |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | Again a comparison is made of the 3 isomers of Nitro-Aniline:<br>ortho= 1290 (1130-1470)<br>meta= 310 (230-420)<br>para= 810 (590-1120) |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>No data concerning the exact method, No of animals, no GLP due to the year of realisation. But the report is still refering to Smyth et all (1962) method and one canthink that 6 animals were used in a range finding study |
| **Flag** | : | non confidential |
| **06.08.2001** | | (56) |

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | = 2350 mg/kg bw |
| **Species** | : | guinea pig |
| **Strain** | : | no data |
| **Sex** | : | no data |
| **Number of animals** | : | |
| **Vehicle** | : | no data |
| **Doses** | : | |
| **Method** | : | other: poor data |
| **Year** | : | 1966 |
| **GLP** | : | no |
| **Test substance** | : | no data |
| **Remark** | : | Before GLPs |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid<br>No data concerning test substance, method, No of animals, no GLP due to the year of realisation. Same paper in Russian. |
| **Flag** | : | non confidential |
| **27.08.2001** | | (57) |

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | = 750 mg/kg bw |
| **Species** | : | other: birds quails? |
| **Strain** | : | no data |
| **Sex** | : | no data |
| **Number of animals** | : | |
| **Vehicle** | : | no data |
| **Doses** | : | |
| **Method** | : | other: no data |
| **Year** | : | 1983 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (4) not assignable<br>Species not related to toxicology and reported in the Environm ent Chapter, purity not known, no GLPs |
| **Flag** | : | non confidential |
| **27.08.2001** | | (61) |

### 5.1.2 ACUTE INHALATION TOXICITY

| | | |
|---|---|---|
| **Type** | : | other: remark on physical state ans maximum theoretical saturating vapour |
| **Value** | : | |

| | | |
|---|---|---|
| **Species** | : | |
| **Strain** | : | |
| **Sex** | : | |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **Doses** | : | |
| **Exposure time** | : | |
| **Remark** | : | 2-nitroaniline is a solid with a melting point of 69-71°Ca, vapour pressure of 0.0037 hPa at 25°C and 1.33 hPA at 104°C.<br>So, there is no indication potential hazard at normal physical state ( flakes) and temperature, but if there is a use at high temperature, exposure could occur according to system used. An assay on repeat administration at vapour state has been run , see in chapter 5.4.<br>Maximum obtainable saturating vapour pressure:<br>(VP (mmHg)/760)x10E6<br>here VP=0.0037hPa= 0.00278 mm Hg then Vp state 25°C= 3.6 ppm and 1 ppm= (24.45/MW)xmg/mE3; then Theoretical Saturating Vapour at 25°C is around 20.7 mg/m3.<br>This far below the recommended dose of 20mg/L. This was only achieved in a repated dose study. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **25.06.2002** | | |

### 5.1.3 ACUTE DERMAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | > 20000 mg/kg bw |
| **Species** | : | rabbit |
| **Strain** | : | New Zealand white |
| **Sex** | : | female |
| **Number of animals** | : | 3 |
| **Vehicle** | : | other: no vehicle |
| **Doses** | : | 20,000 mg/kg |
| **Method** | : | other: method of Smyth etal. (1962) |
| **Year** | : | 1977 |
| **GLP** | : | no |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Method** | : | Undiluted material was applicated to the skin of rabbit trunk using a modification of the rubber cuff of Food and Drug Admistration. The dose is retained under a flexible film of rubber, vinyl plastic or the like, selected to be imperviuos to the chemical. The dosage was 20 ml/kg. |
| **Remark** | : | Before GLPs |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>methodology well described, but no GLPs |
| **Flag** | : | non confidential, Risk Assessment |
| **25.06.2002** | | |

### 5.1.4 ACUTE TOXICITY, OTHER ROUTES

### 5.2.1 SKIN IRRITATION

| | | |
|---|---|---|
| **Species** | : | rabbit |
| **Concentration** | : | 500 other: mg, undiluted |
| **Exposure** | : | Occlusive |
| **Exposure time** | : | 24 hour(s) |
| **Number of animals** | : | |

| | | |
|---|---|---|
| **Vehicle** | : | |
| **PDII** | : | |
| **Result** | : | not irritating |
| **Classification** | : | not irritating |
| **Method** | : | Draize Test |
| **Year** | : | 1977 |
| **GLP** | : | no |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | Before GLPs. The exposure time being more than the one now used in OECD method, the dose being similar : it is assumed that the compound is not irritating to the skin.<br>NO detailed data to indicate the scores. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>No data on number of animals, no GLPs due to the year of realisation. Evaluated by BUA, this report was made available by HOECHST, and is now from Clariant. |
| **Flag** | : | confidential, Risk Assessment |

**25.06.2002** (62)

### 5.2.2 EYE IRRITATION

| | | |
|---|---|---|
| **Species** | : | rabbit |
| **Concentration** | : | undiluted |
| **Dose** | : | 100 other: mg |
| **Exposure time** | : | 24 hour(s) |
| **Comment** | : | not rinsed |
| **Number of animals** | : | |
| **Vehicle** | : | |
| **Result** | : | slightly irritating |
| **Classification** | : | not irritating |
| **Method** | : | Draize Test |
| **Year** | : | 1977 |
| **GLP** | : | no |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | Symptoms disappeared 72 hours after application.<br>No detailed data to indicate the score. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>No data on number of animals, no GLPs due to the year of realisation. Evaluated by BUA, HOECHST report. As for skin irritation this report is now from Clariant.In agreement with actual OECD guideline. |
| **Flag** | : | confidential, Risk Assessment |

25.06.2002 (62)

### 5.3 SENSITIZATION

| | | |
|---|---|---|
| **Type** | : | Guinea pig maximization test |
| **Species** | : | guinea pig |
| **Concentration** | : | 1st: Induction 5 % active substance intracutaneous<br>2nd: Induction 50 % active substance occlusive epicutaneous<br>3rd: Challenge 50 % active substance occlusive epicutaneous |
| **Number of animals** | : | 30 |
| **Vehicle** | : | other: polyethylene glycol 400 |
| **Result** | : | not sensitizing |
| **Classification** | : | not sensitizing |
| **Method** | : | OECD Guide-line 406 "Skin Sensitization" |

| | | |
|---|---|---|
| **Year** | : | 1990 |
| **GLP** | : | yes |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Result** | : | No positive reaction in the 20 tested animals. The summary obtained do not indicate individual score. |
| **Source** | : | Bayer AG Leverkusen<br>Rhodia Recherches Saint Fons |
| **Reliability** | : | (1) valid without restriction<br>no data concerning the exact methodology of the test (concentratrions used, tested product) |
| **Flag** | : | non confidential, Risk Assessment |
| **25.06.2002** | | |
| **Type** | : | Patch-Test |
| **Species** | : | human |
| **Concentration** | : | 1st: Induction 2 % other: patch test<br>2nd:<br>3rd: |
| **Number of animals** | : | 40 |
| **Vehicle** | : | other: yellow paraffin |
| **Result** | : | not sensitizing |
| **Classification** | : | not sensitizing |
| **Method** | : | other: patch test in human |
| **Year** | : | 1975 |
| **GLP** | : | no |
| **Test substance** | : | other TS: product chemically pure |
| **Remark** | : | No GLP for human trials, and study performed before GLPs |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test condition** | : | Investigations were performed on patients with primary contact, atopic, nummular, stasis dermatitis and unclassified eczema. All patients were hypersensitive to p-phenylene-diamine. Patches were applied to the lateral aspect of the arm and the results were read after 48 and 96 hours. Erythema and infiltration were recorded as a positive result even if present only durind the first reading. |
| **Reliability** | : | (4) not assignable<br>no data concerning details, number of patients. Citation. |

27.08.2001 (63)

### 5.4 REPEATED DOSE TOXICITY

| | | |
|---|---|---|
| **Type** | : | Sub-acute |
| **Species** | : | rat |
| **Sex** | : | male |
| **Strain** | : | no data |
| **Route of admin.** | : | inhalation |
| **Exposure period** | : | 6 hours / day / 4 weeks |
| **Frequency of treatm.** | : | 5 days/week |
| **Post exposure period** | : | no data |
| **Doses** | : | 0, 10, 90 mg/m3 |
| **Control group** | : | yes |
| **NOAEL** | : | >= 10 mg/m³ |
| **LOAEL** | : | = 90 mg/m³ |
| **Method** | : | other: few data |
| **Year** | : | 1983 |
| **GLP** | : | no data |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | The compound is certainly at vapour state at 10 mg/m3, theoretical saturating vapour limit at 25°C is around 20.7 mg/m3. But is also at aerosol |

state at dose of 90 mg/m3.

**Result** : Increased tearing and nasal secretions as well as yellowing of the fur were observed in the groups exposed to o-nitroaniline.
Weight gain was unaffected.
In the 90 mg/m3 group, a slight increase of the Met-Hb level and the hematocrit value occured as well as a marginal reduction of leukocytes and segmented neutrophils counts.
The testicular weight was unaffected.
Macroscopic and microscopic examinations of organs showed no indications of substance-caused damage.
Then the dose of 10 mg/kg/day is considered as the NOEL=NOAEL.

**Source** : Rhodia Recherches Saint Fons

**Reliability** : (2) valid with restrictions
not enough details for robuste summary, no data on the method
used, no GLPs, but evaluated by BUA 5 Monsanto/Soluti a report).

**Flag** : confidential, Risk Assessment

**25.06.2002** (64)

**Type** : Sub-acute
**Species** : rat
**Sex** : male/female
**Strain** : Sprague-Dawley
**Route of admin.** : gavage
**Exposure period** : 14 days
**Frequency of treatm.** : Daily 7/7
**Post exposure period** : no data
**Doses** : 0, 1, 10, 100 mg/kg bw
**Control group** : yes, concurrent vehicle
**NOAEL** : >= 100 mg/kg bw
**Method** : other: cf RM
**Year** : 1988
**GLP** : no data
**Test substance** : other TS: from Aldrich, purity 97-99%
**Remark** : 10 rat/sexe/groupe
Examination : behaviour, bodyweight, haematology, biochemistry,histopathology of 28 organs. No effect seen.
vehicle : corn oil

**Source** : Rhodia Recherches Saint Fons

**Reliability** : (2) valid with restrictions
not enough details for robust summary.

**Flag** : non confidential, Risk Assessment

**02.01.2002** (65)

**Type** : Sub-chronic
**Species** : rat
**Sex** : male/female
**Strain** : Sprague-Dawley
**Route of admin.** : gavage
**Exposure period** : 9 weeks. Males : from week 4 prior mating, during mating, gestation of the females. Females : from week 4 prior mating, during mating, gestation and lactation periods until post-partum day 4.(9 weeks approximately)
**Frequency of treatm.** : daily (7days a week)
**Post exposure period** : No
**Doses** : 0, 50, 150, 450 mg/kg bw.
**Control group** : yes, concurrent vehicle
**NOAEL** : >= 50 mg/kg bw
**LOAEL** : = 150 mg/kg bw
**Method** : other: OECD 422 method

| | | |
|---|---|---|
| **Year** | : | 2001 |
| **GLP** | : | yes |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Method** | : | As part of a reprotoxicity study, the number of animals was 12 per sex at the beginning and 10 animals per sex were used to continue the reprotoxicity part of the study and the examinations. |
| **Remark** | : | Vehicle : PEG 400 |
| **Result** | : | The only signs related to treatment were piloerection, salivation and matted fur observed after treatment. Matted fur was also observed and clinical signs performed at weekly intervals in males and females of the high-dose group.<br>No cyanosis was seen as an indication of methemoglobinemia.<br>Statistically significant reduction in body weight (5-6%) were observed at different time in high and mid dose groups during the treatment.<br>A statistically significant reduction in terminal body-weight was observed in high-dose males (6%) compared to controls.<br>No differences were observed in absolute and relative organ weights of males.<br>Macroscopic and microscopic observations of all organs, including spermatogenic cycle, did not reveal any treatment-related effects.<br>The report indicate "The NOEL was established at 150 mg/kg bw/day for for parental and F1 generations".<br>Taking into account the lower bodyweight gain, the NOEL is established at 50 mg/kg bw. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (1) valid without restriction<br>Study according to OECD 422 ( all organs, but not hematology or biochemistry: no indication of MetHb in preliminary study). |
| **Flag** | : | confidential, Risk Assessment |

25.06.2002 (66)

### 5.5 GENETIC TOXICITY 'IN VITRO'

| | | |
|---|---|---|
| **Type** | : | Ames test |
| **System of testing** | : | Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538 |
| **Test concentration** | : | 10 - 5000 µg/plate |
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | negative |
| **Method** | : | other: similar to OECD 471 |
| **Year** | : | 1985 |
| **GLP** | : | no data |
| **Test substance** | : | other TS:purified by recrystallisation |
| **Remark** | : | Comparison of several chemicals among which the 3 isomers of nitroaniline.<br>Test material solvent : DMSO.<br>The author stress up the fact that:<br>"the 3 nitroanilines and the 9 nitroaminophenols are isomers...mutagenicity or non-mutagenicity seems to depend on the position of the electron donating amino ($NH_2$) and hydroxy (OH)groups and the electron accepting nitro ($NO_2$) group in the structure of these compounds"<br>He also emphasises on impurities for differences with Garner and Nutman (1977):<br>"These differences may be due to impurities in the test samples....Mutagenic contaminants are a potential source of false positive results in mutagenicity testing, and it is therefore important that chemical purity be considered in the interpretation of test results."<br>In this case, 1 -chloro-2-nitrobenzene (CAS 88-73-3) have shown a weak |

bacterial mutagenic activity on some Salmonella strains and may account for diffrences.

**Source** : Rhodia Recherches Saint Fons
**Reliability** : (1) valid without restriction
no data on GLPs. The test substance was prepared in the lab and purified by 2 recrystallisation.
Concurrent positive controls. The positivity is based on x2.5 revertant colonies.
Close to OECD Method
**Flag** : non confidential, Risk Assessment, Critical study for SIDS endpoint
**31.08.2001** (58)

**Type** : Ames test
**System of testing** : TA98; TA1538; TA1537; TA100; TA1535;
**Test concentration** : 5; 1; 0.5; 0.1; 0.05 & 0.01 mg/plate
**Cycotoxic concentr.** : 1 mg
**Metabolic activation** : with and without
**Result** : negative
**Method** : other: like OECD Guide-line 471
**Year** : 1986
**GLP** :
**Test substance** : other TS: >99%
**Result** : ortho-nitroaniline is negative, while over the 35 compound reported , in some s trains (TA98 &TA1538) para-nitroaniline showed a weak effect and meta-nitroaniline is positive.
**Source** : Rhodia Recherches Saint Fons
**Reliability** : (1) valid without restriction
No GLP's reported, otherwise very similar to OECD 471
**Flag** : non confidential, Risk Assessment, Critical study for SIDS endpoint
**27.08.2001** (67)

**Type** : Ames test
**System of testing** : Salmonella typhimurium TA98, TA100
**Test concentration** : 2500 µg/plate
**Cycotoxic concentr.** : no data
**Metabolic activation** : with
**Result** : negative
**Method** : other: accpording to Ames (1975) and Maronet al.(1983)
**Year** : 1985
**GLP** : no data
**Test substance** : as prescribed by 1.1 - 1.4
**Remark** : Activation system : S9 Hamster and many others(rat; mouse, dog and including Man).
2-nitroaniline is reported only weakly positive in presence hamster S9 on the strain TA 98 (used for frameshift mutation), while negative in all other tests systems.
This indicate that positive result with hamster S9 is not relevant for man which showed the same result as the other species tested among which the rat.
**Source** : Rhodia Recherches Saint Fons
**Reliability** : (2) valid with restrictions
The study is done as in OECD guideline but only two tester strain
**Flag** : non confidential, Risk Assessment
**27.08.2001** (68)

**Type** : Ames test
**System of testing** : Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, D3052
**Test concentration** : 1000 µg/plate
**Cycotoxic concentr.** : no data

| | | |
|---|---|---|
| **Metabolic activation** | : | with and without |
| **Result** | : | negative |
| **Method** | : | other: modified Ames gradient plate (McMahon, 1979) |
| **Year** | : | 1983 |
| **GLP** | : | no data |
| **Test substance** | : | other TS: Aldrich reagent grade |
| **Remark** | : | A total of 45 compounds were testedand the 3 isomers were compared. Ortho –nitroaniline is the only negative isomer while the 2 others isomers show some positivity according to the strains, as previoulsly seen in Shimizu(1986). This assay was also done together with E.coli and UDS (negative)as reported after. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions the concentrations tested are not reported in this assay which is using a diffusion from a disk including 1000 µg material. Not an OECD method, but using positive controls and comparing many chemicals. |
| **Flag** | : | non confidential, Risk Assessment |
| **27.08.2001** | | (69) |

| | | |
|---|---|---|
| **Type** | : | Ames test |
| **System of testing** | : | Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538, TA97 |
| **Test concentration** | : | no data |
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | negative |
| **Method** | : | other: Method according to Ames |
| **Year** | : | 1984 |
| **GLP** | : | no data |
| **Test substance** | : | other TS: reagent pure grade |
| **Result** | : | 135 chemicals were tested, and also on E.coli DNA repair. The only indication is the Potency (revertants per nanomole: <0.002). Some are used as positive controls. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid no data on the concentrations tested, no GLPs, but cited in BUA |
| **Flag** | : | non confidential, Risk Assessment |
| **07.08.2001** | | (70) |

| | | |
|---|---|---|
| **Type** | : | Ames test |
| **System of testing** | : | Salmonella typhimurium TA97, TA102 |
| **Test concentration** | : | no data |
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | negative |
| **Method** | : | other: according to Ames |
| **Year** | : | 1984 |
| **GLP** | : | no data |
| **Test substance** | : | other TS: reagent pure grade |
| **Remark** | : | Solvent : DMSO |
| **Result** | : | same result as in the previous paper. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid No tested concentrations, no GLPs, 2 strains only, not in compliance with OECD method, but cited in BUA |
| **Flag** | : | non confidential |
| **06.08.2001** | | (71) |

| | | |
|---|---|---|
| **Type** | : | Ames test |
| **System of testing** | : | Salmonella typhimurium TA98, TA100 |

| | | |
|---|---|---|
| **Test concentration** | : | 0.1, 1, 1 0 µmole/2 ml agar (10 µM or 13,8 mg/ 2ml or 6,9 mg/ml) |
| **Cycotoxic concentr.** | : | > 10 µmole or 6,9 mg/ml |
| **Metabolic activation** | : | without |
| **Result** | : | negative |
| **Method** | : | other: according to Ames |
| **Year** | : | 1977 |
| **GLP** | : | no |
| **Test substance** | : | other TS: from Eastman Chemicals |
| **Remark** | : | 53 compounds were tested and some used as positive controls. The 3 isomers were tested and only meta-nitroaniline showed positive results on TA98. solvent = DMSO |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions before GLP's.No data on the purity of the test substance, although from known company. 2 strains only,not in compliance with OECD method, but similar in principle. |
| **Flag** | : | non confidential, Risk Assessment |
| **27.08.2001** | | (72) |

| | | |
|---|---|---|
| **Type** | : | Ames test |
| **System of testing** | : | Salmonella typhimurium TA98, TA100 |
| **Test concentration** | : | no data |
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | negative |
| **Method** | : | other: few indications |
| **Year** | : | 1987 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Remark** | : | 102 chemicals were tested, among which the 3 nitroaniline isomers. Only meta-nitroaniline showed poistive results on both s trains W/O metabolic activation. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid no data on the test substance, the concentrations tested and the exact methodology. 2 strains only, not in compliance with OECD method. Text in Japanese, only tables could be assessed. |
| **Flag** | : | non confidential |
| **06.08.2001** | | (73) |

| | | |
|---|---|---|
| **Type** | : | Ames test |
| **System of testing** | : | Salmonella typhimurium TA1538 |
| **Test concentration** | : | 50, 100 µg/plate |
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | ambiguous |
| **Method** | : | other: according to Ames |
| **Year** | : | 1977 |
| **GLP** | : | no data |
| **Test substance** | : | other TS: from Aldrich chem. company, no purity mentionned |
| **Remark** | : | before GLPs. These results of positivity: meta<ortho<para is different from several other studies where it was ortho<para<meta, with genarally no mutagenicity for ortho, weak on some strains for para and more evident mutagenicity for meta, it is the only one indicating positivity with strain TA1538? (The author have just made purification for 2 crude dyes toconfirm their mutagenic activity) Solvent : DMSO |
| **Result** | : | Ten Azo-dyes were studied including the 3 nitroaniline isomers. Ortho |

nitroaniline is negative without activation, positive with activation? The order in thiss study is :meta<orhto<p

**Source** : Rhodia Recherches Saint Fons
**Reliability** : (2) valid with restrictions
No GLPs, purity not specified. One strain o nly, not in compliance with OECD method. Results in disaccordance with other studies on comparison of the 3 isomers.
**Flag** : non confidential, Risk Assessment
**27.08.2001** (74)

**Type** : Ames test
**System of testing** : Salmonella typhimurium TA98, TA100
**Test concentration** : 0 to 10 µmol/plate (6900µg/plate)
**Cycotoxic concentr.** : 10 µM or 6900 mg
**Metabolic activation** : with and without
**Result** : negative
**Method** : other: According to Ames, with 30 mn preincubation without shakingand use of FMN(flavin mononucleotide) cofatctor
**Year** : 1989
**GLP** : no data
**Test substance** : other TS: Aldrich purity 98%
**Remark** : Solvent : p-dioxane
With and w ithout metabolic activation : S9 rat with Flavin Mononucleotide (FM), or S9 hamster without FM
**Result** : Results negative with or without activation, but one positive result( only high cytotoxic dose: around x2) with activation (S9 Hamster) and FMN cofacto r, but not with Hamster S9 and neither with Rat S9, and is also ctytoxic at 10 µM.
In the same study, metanitroaniline (97%) purity was dose-dependent positive with FMN+rat or Hamster S9; paranitroaniline (>99%) was positive in all cases. We consider the result of ortho nitroaniline as NEGATIVE by comparison of the graphs of the 2 other isomers and other data obtained in normal methods.
**Source** : Rhodia Recherches Saint Fons
**Reliability** : (2) valid with restrictions
no GLPs, 2 strains only, not in compliance with OECD method.Special activation system with Flavin mononucleotide.
**Flag** : non confidential, Risk Assessment
**27.08.2001** (75)

**Type** : Ames test
**System of testing** : Salmonella typhimurium TA97, TA98, TA100, TA102
**Test concentration** : 1 - 1000 µg/plate
**Cycotoxic concentr.** : no data
**Metabolic activation** : with and without
**Result** : negative
**Method** : other: no data
**Year** : 1994
**GLP** : no data
**Test substance** : no data
**Source** : Rhodia Recherches Saint Fons
**Reliability** : (2) valid with restrictions
no data on the test substance, study published recently in a worldwide journal. These data are in good agreement with the most reliable ones.
**Flag** : non confidential, Risk Assessment
**07.08.2001** (76)

**Type** : Ames test
**System of testing** : Salmonella typhimuri um TA98, TA100
**Test concentration** : 174 - 2515 µg/plate

| | | |
|---|---|---|
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | negative |
| **Method** | : | other: no data |
| **Year** | : | 1997 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>no data on the test substance but recent study published in a worldwide journal. Two strains only, not in compliance with OECD method, but similar to it. These data are in agreement with the most reliable ones. |
| **Flag** | : | non confidential, Risk Assessment |
| **07.08.2001** | | (77) |

| | | |
|---|---|---|
| **Type** | : | Bacillus subtilis recombination assay |
| **System of testing** | : | Bacillus subtilis H17, M45 |
| **Test concentration** | : | 500 - 5000 µg/plate |
| **Cycotoxic concentr.** | : | no cytoxicity |
| **Metabolic activation** | : | without |
| **Result** | : | ambiguous |
| **Method** | : | other:method described by Kada |
| **Year** | : | 1986 |
| **GLP** | : | no data |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Result** | : | Result is evaluated by inhibition of recombinant of strains M45(Rec-) and H17(Rec+) and a difference of 1 mm is considered as a positive response.<br>The 3 isomers were negative at 0.5 mg but positive at 5 mg.<br>There is no indication of cytotoxicity and at this dose orthonitroaniline is the only isomer totally inhibiting Salmonella growth?<br>It is then difficult to clearly qualify the result positive.<br>Rec Assay is declare "generally giving more positive results than Ames test." |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>no GLPs, method not in compliance with OECD guidelines. No indication of cytotoxicity.No clear way to understand reliabilty, but taken as 2 due to the Ames part of the paper. |
| **Flag** | : | non confidential, Risk Assessment |
| **27.08.2001** | | (67) |

| | | |
|---|---|---|
| **Type** | : | Escherichia coli reverse mutation assay |
| **System of testing** | : | Escherichia coli WP2uvrA, WP2 |
| **Test concentration** | : | 0.6 - 100 µg/ml |
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | negative |
| **Method** | : | other: according to OECD 412 |
| **Year** | : | 1983 |
| **GLP** | : | no data |
| **Test substance** | : | other TS: reagent pure grade |
| **Remark** | : | solvent : DMSO |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions<br>No GLPs |
| **Flag** | : | non confidential, Risk Assessment |
| **06.08.2001** | | (69) |

| | | |
|---|---|---|
| **Type** | : | Escherichia coli reverse mutation assay |
| **System of testing** | : | E. coli WP2uvrA/pKM101 |

| | | |
|---|---|---|
| **Test concentration** | : | no data |
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | negative |
| **Method** | : | other: accordind to Ames |
| **Year** | : | 1987 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Remark** | : | Metabolic activation : S9 rat<br>Test with preincubation, with and without metabolic activation |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (4) not assignable<br>no data on the test substance, the concentrations tested and the exact methodology, Language Japanese |
| **Flag** | : | non confidential |
| **27.08.2001** | | (73) |

| | | |
|---|---|---|
| **Type** | : | Escherichia coli reverse mutation assay |
| **System of testing** | : | Escherichia coli WP2uvrA, WP2uvrA/pKM |
| **Test concentration** | : | no data |
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | positive |
| **Method** | : | other: few data |
| **Year** | : | 1983 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Remark** | : | no correspondance with the reference |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid<br>no data on the test substance, the concentrations tested and the exact methodology. Method not in compliance with OECD guidelines. |
| **Flag** | : | non confidential |
| **27.08.2001** | | (78) |

| | | |
|---|---|---|
| **Type** | : | Escherichia coli reverse mutation assay |
| **System of testing** | : | Escherichia coli WP2, WP67, CM871 |
| **Test concentration** | : | no data |
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | ambiguous |
| **Method** | : | other: Kada |
| **Year** | : | 1984 |
| **GLP** | : | no data |
| **Test substance** | : | other TS: reagent grade pure |
| **Remark** | : | solvent : DMSO |
| **Result** | : | Ortho-nitroaniline is just reported in a graph, as C2 compound, where is done a comparison of compound positive in 1 test, and C2 was negative in Ames with a very low potency of revertant /nmole( <0.0005)?<br>See the next paper of the same group . |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid<br>No tested concentrations, no GLPs, not in compliance with OECD guidelines but cited and evaluated in BUA |
| **Flag** | : | non confidential |
| **27.08.2001** | | (79) |

| | | |
|---|---|---|
| **Type** | : | Escherichia coli reverse mutation assay |
| **System of testing** | : | Escherichia coli WP2, WP67, CM871; and Samonella TA97, TA102 |
| **Test concentration** | : | no data |

| | | |
|---|---|---|
| **Cycotoxic concentr.** | : | no data |
| **Metabolic activation** | : | with and without |
| **Result** | : | negative |
| **Method** | : | other: Kada |
| **Year** | : | 1984 |
| **GLP** | : | no data |
| **Test substance** | : | other TS: reagent pure grade |
| **Remark** | : | Solvent : DMSO |
| **Result** | : | Again the result is only expressed as potency of DNA-repair: it is 0.027 witout S9 and 0 with S9. They were also tested on Salmonelle TA97and TA 102; o-nitroaniline was negative |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid<br>No tested concentrations, no GLPs, not in compliance with OECD guidelines but cited and evaluated in BUA |
| **Flag** | : | non confidential, Risk Assessment |
| **27.08.2001** | | (71) |

| | | |
|---|---|---|
| **Type** | : | Micronucleus test in vitro |
| **System of testing** | : | Micronucleus test in Chinese hamster lung cell line (CHL/IU) |
| **Test concentration** | : | 46-4100 µg/ml (from 3.3E-6 around 3.10E-2 M) |
| **Cycotoxic concentr.** | : | not mentioned (> 800 µg/ml ?) (= 6.10-3 M) |
| **Metabolic activation** | : | with and without |
| **Result** | : | positive |
| **Method** | : | other |
| **Year** | : | 1999 |
| **GLP** | : | no data |
| **Test substance** | : | other TS |
| **Method** | : | Chemicals was suspended in DMSO, immediately before treatment. The cells were treated continously for 24 and 48 hours in absence of S9mix and 6h with S9mix followed by 42h recovery time (indicated as 6+42h = 48h). Cells were detached by trypsinisation and treated with KCl hypotonic solution (75mM) for 10 mn. The cells were then fixed by at least 3 changes of 1:3 acetic acid:ethanol. Finally the cells were suspended in methanol containing 1-2% acetic acid and air dried. The cells were stained with either acridine orange or Giemsa.<br>The number of micronucleus per 1000 intact interphase cells was recorded.<br>Statistical procedure : the frequencies of cells with type 2 and/or type 3 MicroNuclei in the treated groups were compared with those of the current negative control by Fisher's exact test. The concentration response relationship was evaluated by Cochran-Armitage trend test.<br>Result statistically significant when the P value was smaller then 0.05. |
| **Remark** | : | Validation study to prepare a new Japanese guideline.Concentration extremely high 3.10-2 M and cytotoxicity not mentionned. This guideline is not yet validated. |
| **Result** | : | The compound induced polyploid cells with 24 and 48 h continuous treatments.<br>In the 24h treatment, a marginal response (9%) was seen in Chromosomal Aberrations at the lower conc. (130 µg/ml).<br>In the 48h treatment test a dose-dependant response was seen (7-22% at 130-250 µg/ml respectively).<br>Short treatment :<br>without S9, induced polypoid cells without dose response relationship, and structural aberrations were observed at 800 µg/ml (18.7%).<br>with S9, induced structural aberrations with dose dependency (10-35.4% at 200-800 µg/ml respectively). |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Test substance** | : | producer: Wako Pure Chemical Industries Ltd., Osaka, Japan |
| **Reliability** | : | (2) valid with restrictions<br>No data concerning the GLP, method not in compliance with the OECD |

guidelines and not yet a Japanese guideline. No indication of cytotoxicity while using very high doses compared to other in vitro systems, all positive data are seen between 130-800 µg/ml( 1 to 6xmMole). These values are in contrast with the cytotoxic concentration noted for rat hepatocytes 1 mM (138 µg/ml)or 50 nM (0.079 µg/ml)? This does not seems realistic values even looking at Bacterial cytotoxicity. Finally such an in vitro result is not supported by in vivo data.

**Flag** : non confidential, Risk Assessment
**27.08.2001** (80)

**Type** : Unscheduled DNA synthesis
**System of testing** : Rodent hepatocytes
**Test concentration** : 10-3 to 10-6 M
**Cycotoxic concentr.** : 10-3 M (138 µg/ml)
**Metabolic activation** :
**Result** : negative
**Method** : other: Williams et al.
**Year** : 1988
**GLP** : no data
**Test substance** : other TS: reagent pure grade
**Remark** : 37 aniline derivatives were tested. Of which 6 gave positive results which are in agreement with bacterial mutagenicity with or witout Norhaman. Three were of unknown carcinogenicity.
**Source** : Rhodia Recherches Saint Fons
**Reliability** : (2) valid with restrictions
no GLPs
**Flag** : non confidential, Risk Assessment
**27.08.2001** (81)

**Type** : Unscheduled DNA synthesis
**System of testing** : Rat hepatocytes
**Test concentration** : 8 concentrations : 0.5 - 1 000 nMole/ml (extremely low concentrations: up to 1.38 µg/ml)
**Cycotoxic concentr.** : > 50 nmol/ml ( 0.079 µg/ml)
**Metabolic activation** :
**Result** : negative
**Method** : other: according to Williams method
**Year** : 1983
**GLP** : no data
**Test substance** : other TS: reagent pure grade
**Method** : Primary cultures of adult rat hepatocytes were prepared by in situ perfusion of liver freom 150-170 g male Fisher 344 rats by the method of Williams et al. (1977) and conducted as described by Probst et al.(1981).
8 concentrations were tested over the range of 1000-0.5 nM/ml (0,13 µg/ml...)
**Remark** : solvent : no data. Extremely low concentrations, this is repeated in the text and tables.
**Result** : Starting at the cytotoxic concentration of 50nM/ml (0.079 µg/ml) orthonitroaniline is negative, as well as the 2 other isomers which were cytoytoxic at 500 nM.
**Source** : Rhodia Recherches Saint Fons
**Reliability** : (2) valid with restrictions
no GLPs indicated and low concentrations indicated(?) but done according to guidelines.
**Flag** : non confidential, Risk Assessment
27.08.2001 (69)

## 5.6 GENETIC TOXICITY 'IN VIVO'

| | | |
|---|---|---|
| **Type** | : | Micronucleus assay |
| **Species** | : | mouse |
| **Sex** | : | no data |
| **Strain** | : | no data |
| **Route of admin.** | : | i.p. |
| **Exposure period** | : | no data |
| **Doses** | : | 50, 250, 500 mg/kg bw |
| **Result** | : | negative |
| **Method** | : | other: few data |
| **Year** | : | 1989 |
| **GLP** | : | no data |
| **Test substance** | : | other TS: Monsanto? |
| **Remark** | : | This is in support of other in vivo studies but need details for a better reliability assessment. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (3) invalid no data on the tested substance and the exact methodology, no GLPs. (need for Monsanto/ Solutia report for more details) |
| **Flag** | : | confidential, Risk Assessment |

**27.08.2001** (82)

| | | |
|---|---|---|
| **Type** | : | other: DNA damages - alkaline elution |
| **Species** | : | mouse |
| **Sex** | : | male |
| **Strain** | : | CD-1 |
| **Route of admin.** | : | i.p. |
| **Exposure period** | : | 4 hours |
| **Doses** | : | 100 mg/kg bw |
| **Result** | : | negative |
| **Method** | : | other: DNA damages were evaluated by the elution technique coupled with a microfluorimetric method for DNA assay |
| **Year** | : | 1982 |
| **GLP** | : | no data |
| **Test substance** | : | no data |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (2) valid with restrictions no data on the test substance, no GLPs, but evaluated by BUA report.This assay in agreement with guidelines. |
| **Flag** | : | non confidential, Risk Assessment |

**27.08.2001** (83)

| | | |
|---|---|---|
| **Type** | : | Micronucleus assay |
| **Species** | : | mouse |
| **Sex** | : | male/female |
| **Strain** | : | NMRI |
| **Route of admin.** | : | i.p. |
| **Exposure period** | : | 16, 24 and 48 hours after administration |
| **Doses** | : | 500 mg/kg bw |
| **Result** | : | negative |
| **Method** | : | OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test" |
| **Year** | : | 1993 |
| **GLP** | : | yes |
| **Test substance** | : | other TS: purity 65% (water 29.5%) |
| **Remark** | : | The IUCLID indicated OECD 474. We do not have all details of this report. It is assumed to be done with 5 animals and the dose of 500 mg/kg i.p. is the high dose of the Monsanto study, for which we do not have the report, but |

the same negative results.
With the 3 times studied (16, 24 and 48 hours) the possibility to get micronuclei is pretty well coverded.

**Source** : ECB IUCLID
**Reliability** : (1) valid without restriction
**Flag** : confidential, Risk Assessment, Critical study for SIDS endpoint
**25.06.2002** (84)

### 5.7 CARCINOGENICITY

### 5.8.1 TOXICITY TO FERTILITY

| | | |
|---|---|---|
| **Type** | : | other: OECD 422 method |
| **Species** | : | rat |
| **Sex** | : | male/female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | gavage |
| **Exposure period** | : | Males : from 4 weeks prior to mating, during mating, gestation of the females : i.e: 9 weeks.<br>Females : from 4 weeks prior to mating, during mating, gestation and lactation periods until post-partum day 3. Approximately 9 weeks |
| **Frequency of treatm.** | : | Daily (7 days a week) |
| **Premating exposure period** | | |
| **Male** | : | 4 weeks |
| **Female** | : | 4 weeks |
| **Duration of test** | : | 9 weeks. Males : from week 4 prior mating, during mating, gestation of the females. Females : from week 4 prior mating, during mating, gestation and lactation periods until post-partum day 4.(9 weeks approximately) |
| **No. of generation studies** | : | |
| **Doses** | : | 0, 50, 150, 450 mg/kg bw |
| **Control group** | : | yes, concurrent vehicle |
| **NOAEL parental** | : | = 50 mg/kg bw |
| **NOAEL F1 offspring** | : | = 50 mg/kg bw |
| **LOEL parental** | : | = 150 mg/kg bw |
| **LOEL F1 offspring** | : | = 150 mg/kg bw |
| **Result** | : | No effect at non maternal toxic dose |
| **Method** | : | other: OECD 422 |
| **Year** | : | 2001 |
| **GLP** | : | yes |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Method** | : | As the reprotoxicity part of the OECD 422 guideline, 12 nulliparus females and 12 males were used up to the gestation period were 10 animals were followed as requested by the method. |
| **Result** | : | Parental clinical observation:as in Repeat part,<br>The only signs related totreatment were piloerection, salivation and matted fur observed at post-dose observations. Matted fur was also observed at clinical signs performed at weekly intervals in males and females of the high-dose group.<br>No indication of cyanosis was noted as a parameter for hematotoxicity (MetHemoglobin formation).<br><br>Reproductive parameters : The copulatory and fertility index, as well as the pre-coital intervals, were not affected by treatment. Implantation and pre-birth loss were unaffected by treatment. |

Parental body weights : Statistically significant reduction in body weight were observed at several weighing time in high- and mid-dose groups (males and females: 5 to 6%) during the treatment. A significant reduction in terminal body-weight or bodyweight gain was observed in high-dose males (6%) compared to controls, and more important in dams on gestation day 20 (bwg: -15%) and on day +4 post-partum ( weight loss in 5 females)in high-dose females.

This have a direct effect on pups ( post-partum deaths were seen in dams with lower bwg at day 20 or loss at day +4): an increased incidence in the number of pups found dead was observed between days 0 and 2 post partum in the high dose, with a significant increase of male pup deaths.
Necropsy findings in decedent pups : the findings observed at necropsy in decedent pups were similar in the control and the treated groups.
Necropsy findings in F1 pups at day 4 post-partum: in general ther were no particular differences between control and treated groups, with the exception of 2 pups each in the mid- and high-dose groups that showed abnormal size of the median lobe of the liver in association with an abnormal area and abnormal color.

Parental terminal organ weights : No differences were observed in absolute and relative organ weights of male parents.
Macroscopic and microscopic observations of parental generation : macroscopic and microscopic examinations of all organs, including spermatogenic cycle, did not reveal any treatment-related effects.
Control and treted females showed persistent corpora lutea whcih was considered to be a physiological condition during lactation.

**Source** : Rhodia Recherches Saint Fons
**Reliability** : (1) valid without restriction
Study according to OECD 422; long male treatment to have 9 weeks exposure, like female.Organs were examined as in 422 but no bioachemistry or hematology was measured.
**Flag** : confidential, Risk Assessment
28.10.2002 (66)

### 5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

| | | |
|---|---|---|
| **Species** | : | rat |
| **Sex** | : | female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | gavage |
| **Exposure period** | : | from day 6 to day 15 of the gestation |
| **Frequency of treatm.** | : | daily |
| **Duration of test** | : | Autopsy of the animals and caesarean section on the 21st gestation day |
| **Doses** | : | 50, 200, 400, 800, 1200 mg/kg |
| **Control group** | : | yes, concurrent vehicle |
| **NOAEL maternal tox.** | : | = 400 mg/kg bw |
| **NOAEL teratogen.** | : | = 400 mg/kg bw |
| **LOAEL Fetotoxicity** | : | = 800 mg/kg bw |
| **Result** | : | No developmental or teratogenic effect. |
| **Method** | : | other: pilot teratogenicity study in rats |
| **Year** | : | 1984 |
| **GLP** | : | no data |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | 6 mated females per group<br>vehicle = corn oil - dose volume : 10 ml/kg |

| | | |
|---|---|---|
| **Result** | : | Clinical observations in rats in the two highest groups includes hyperactivity, convulsions, salivation, prostration, piloerection, shallow respiration and loss of muscle coordination and mortality at 1200 mg/kg bw (4/6). A decrease in mean maternal body weight gains was observed in the 800 and 1200 mg/kg dose groups for the gestation interval 6 -12, however it was higher than controls during the entire gestation interval days 6-21. Mean maternal body weight gains in the other groups were comparable to controls. The number of viable foetuses , total implantations, resorptions and fetal malformations was comparable in all dose groups. Mean fetal body weights were comparable in all dose groups, except the 2 highest dose groups in which a decrease was observed. |
| **Source** | : | EPA report NTIS |
| **Reliability** | : | (2) valid with restrictions<br>no GLPs, but evaluated by BUA and TSCA, done according to guideline as a range-finding study. |
| **Flag** | : | non confidential, Risk Assessment |
| **02.01.2002** | | (85) |

| | | |
|---|---|---|
| **Species** | : | rat |
| **Sex** | : | female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | gavage |
| **Exposure period** | : | from day 6 to day 15 of the gestation |
| **Frequency of treatm.** | : | daily |
| **Duration of test** | : | Autopsy of the animals and caesarean section on the 21st gestation day |
| **Doses** | : | 0, 100, 300, 600 mg/kg bw/d |
| **Control group** | : | yes |
| **NOAEL maternal tox.** | : | = 100 mg/kg bw |
| **NOAEL teratogen.** | : | = 300 mg/kg bw |
| **NOAEL Embryotoxicity** | : | = 300 mg/kg bw |
| **Result** | : | No effect at non maternal toxic doses. |
| **Method** | : | other: guideline not specified, but protocol close to the current guidelines |
| **Year** | : | 1985 |
| **GLP** | : | no data |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | 25 females per group<br>Vehicle: corn oil - Dose volume 10 ml/kg |
| **Result** | : | Maternal toxicity was evident by statistical differences between dosed groups and controls for: number of cases of piloerections (mid and high dose groups), mean maternal body weights and food consumption(mid and high dose groups: 6-7%).<br>Pregnancy rate and the number of live and dead fetuses, early and late resorptions, total nidations and corpora lutea were comparable for all groups. No meaningful differences in the total number of litters of fetuses exhibiting malformations was evident. However, one fetus in each two litters at the 600 mg/kg level exhibited partial situs inversus and similar heart malformations. |
| **Source** | : | Report from US EPA |
| **Reliability** | : | (2) valid with restrictions<br>no data on the GLPs, but evaluated by BUA and TSCA. |
| **Flag** | : | non confidential, Risk Assessment |
| **25.06.2002** | | (85) |

| | | |
|---|---|---|
| **Species** | : | rat |
| **Sex** | : | female |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | gavage |
| **Exposure period** | : | Day 0 to day 19 of gestation |
| **Frequency of treatm.** | : | daily |
| **Duration of test** | : | gestation of the animals |

| | | |
|---|---|---|
| **Doses** | : | 100, 200, 400 mg/kg bw |
| **Control group** | : | yes, concurrent vehicle |
| **NOAEL maternal tox.** | : | = 200 mg/kg bw |
| **NOAEL teratogen.** | : | >= 400 mg/kg bw |
| **NOAEL Embryotoxicity** | : | >= 400 mg/kg bw |
| **Method** | : | other: preliminary study before OECD 422. Examination as in OECD 414 |
| **Year** | : | 2001 |
| **GLP** | : | yes |
| **Test substance** | : | as prescribed by 1.1 - 1.4 |
| **Remark** | : | vehicle polyethylene 400 |
| **Result** | : | The only signs attribuable to treatment were matted fur and piloerection seen in animals receiving 400 mg/kg/day. Slight dose-dependant decreases in body weight were noticed in mid-and high-dose animals, but these changes were not statistically significant. No indication of cyanosis( metHb) was noted.<br>A statistically significant reduction in body weight gain was observed in the high-dose group on gestation Days 6 and 20, when compared to controls. There were no differences in uterus and corrected body weights between the control and the treated groups. No signs of toxicological significance were observed in litter data and sex ratios between the control and the treated groups. Macroscopic examinations of females and external foetal examination did not show any treatment related effects. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (1) valid without restriction |
| **Flag** | : | confidential, Risk Assessment |

**25.06.2002** (86)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

| | | |
|---|---|---|
| **Type of experience** | : | other: genral consideration on human toxicity |
| **Remark** | : | Human toxicity |
| **Result** | : | According to Hamblin (1963), cited in BUA, o-nitroaniline has practically the same toxicity in human as p -nitroaniline. The main symptoms of a p-nitroaniline are headaches, reddening of the face, difficult breathing, nausea and vomiting. |
| **Source** | : | Rhodia Recherches Saint Fons |
| **Reliability** | : | (4) not assignable<br>very old publication concerning an other product, no data concerning the route of administration, the number of observations. |
| **Flag** | : | non confidential |

**03.01.2002** (87)

| | | |
|---|---|---|
| **Type of experience** | : | other: comparative study on methemoglobinemia induction |
| **Method** | : | IN VIVO:<br>- Rats: wistar male and female weighting around 250 g received 2 oral administrations at 24h intervals. Blood is taken at orbital sinus at 5 hours after the last administration. 10 rats are used by group and compared to controls.<br>- Dogs: mongrel dogs are usedand treated as rats but with capsules. Blood is taken on heparin at cephalic vein at appropriate times and finally 24h after the last administration.<br>IN VITRO: |

All dosages of:
MetHemoglobin (MetHb) and SulfHemoglobin (SHb) are done according to described methods ( Evelyn et al 1938, De Traverse et al 1961). MetHb is transformed in CyanHb and optical density difference is measured by suppressing the charecteristic absorption of Hb at 635 mμ. SulfHb is measured by residual optical density at 620 mμ after conversion of Hb and MetHb in cyan Hb. Total Hb is measured with Rabkin reagentwith Crosby method.

**Remark** : These results are in good agreement with what has been seen in rats studies wer no cyanosis was identified by oral route even in repeated administration at doses as high as 450 mg/kg bw for 9 weeks.
It must be also mentioned that the authors indictae that dog is more suceptible than rat, and rat more than human.

**Result** : We will only report here dog data concerning trifluoromethyl- aniline isomers(o-m-p TFMA) used at 110-55 or 27.5 mg/kg and aniline at equimolar dose of Aniline: 100 mg/kg in dogs.
First of all, it must be mentionned that dogs are more sensitive to MetHb agents than rats ( Lester-1943 and Spicer-1950).
With paraTFMA after the first administration the animal get a rapid rise in MehHB ( 50% at 1h30) and died within hours. then the other isomers were only admistered at 55x2 mg/kg at this dose MetHb was:
paraTFMA: 49%
metaTFMA: 15%
orthoTFMA: 0%
aniline at 100x2 mg/Kg indicate 46% MetHb.
So pTFMA is more potent MetHb inducer than Aniline, while meta is lower and ortho not inducing.

**Reliability** : (2) valid with restrictions
Old study but correctly discribed and important in MetHb inducion comparing animals and Human, and isomers for induction.

03.01.2002 (88)

### 5.11 ADDITIONAL REMARKS

**Type** : other: hematotoxicity

**Result** : Dinitrobenzene (para-isomer: 29.5%) and m-chloronitrobenzene((31.9%) are the most potent methaemoglobin formers in the rat.
A significant increase of methemoglobinemia (14.2 % at 100 μmole/kg or 13.8 mg/kg i.p.) was observed in vivo, in rats treated with o-nitroaniline.
In vitro, the effect of o-nitoaniline (5.7%) is at the limit of significance ($p < 0.05$)vs controls at 4.2%.

**Source** : Rhodia Recherches Saint Fons

**Test condition** : Products were administered once, by IP route, to Wistar rats at the dose level of 100 μmol/kg ( 13.8 mg/kg for orthonitroaniline. The animals were killed 5 hours after the injection to examine methemoglobin levels.
In vitro formation of methemoglobin was studied by incubating 0.1 μmole of hemoglobin (obtained from control rats) with 0.5 μmole of each tested compounds at pH 6.6 and 37°C for 5 hours.

**Reliability** : (3) invalid
no data on the purity of the product studied, few data on the methodology

**Flag** : non confidential, Risk Assessment

**16.07.2001** (89)

**Type** : other: hematotoxicity

**Result** : The direct acting agents, ranked from most to least potent inducers of methemoglobin formation are : p-dinitrobenzene > o-dinitrobenzene > copper = nitrite > chlorite > chlorate. The ranking from most to least potent inducers of the bioactivated agents are : a-naphtol > p-nitroaniline >

m-nitroaniline, o-nitroaniline > p-nitrotoluene = aniline > m-nitrotoluene = o-nitrotoluene.

**Source** : Rhodia Recherches Saint Fons
**Test condition** : Six agents that ares direct-acting and eight that require bioactivation were tested for their ability to induce methemoglobin formation in Dorser sheep erythrocytes under defined in vitro conditions.
The agents were the ranked according to three complementary methods based on the slope of the linear regression, the calculated dose expected to induce a given amount of methemoglobin formation and the calculated percentage methemoglobin response induced by 1 mmol/l of the agent.
**Reliability** : (2) valid with restrictions
Purity of the tested products unknown
**Flag** : non confidential, Risk Assessment
**27.08.2001** (90)

**Type** : other: Hematotoxicity and structure activity
**Result** : A comparative study was done on dogs, both sexes with several substances among with the 3 ortho- meta- and para-isomers of trifluoromethylaniline. Dog is more sensitive to methaemoglobinemia (MetHb) than rat which is also more sensitive than humans. Blood was taken at the cephalic veinon heparin. After a control sample, dogs were administered orally twice at 24 hours interval substances into capsules. A sample blood was taken 24 hours later. MetHb inducing substances are also leading to sulphaemoglobin (SHb) with SH2 absorbed trough gut due decreased fermentation and transit. MetHb and SHb were measured by optical density method. méthémoglobine et de sulfhémoglobine ont été effectués par mesure de densité optique.
- p-TFMA, lead to a rapid and high level of MetHb and death arrive within 2 hours after the first capsule ingestion.
- with m-TFMA, a high level(30%) is observed within 4 hours after the first ingestion, but recoveru to Hb is also more rapid. For SHb a level <1% is observed after 24 hours after the first ingestion. Sample taken 24 or 72 hours after the second ingestion lead to a total inactivated Hb of 10% representing equal levels of MetHb and SHb.
- with o-TFMA, a low level< 2% appear 4 hours after the first ingestion and is replaced by SHb (1%) after the second ingestion. In the sensitive dog species a dose of 100 to 150 mg/kg is required to reach a 20% MetHb after the 1$^{st}$ ingestion, decreasing rapidly to leave a 2% SHb after 24 hours.
It is concluded that the trifluormethyl (TFM) substitution in para and to a lesser extent in meta increase the methaemoglobimemic potency of aniline, altough recovery to Hb is quick. This is not the case of ortho TFM, a potential hydrogen bond could block the amine effect.
**Source** : Rhodia Recherches Saint Fons
**Reliability** : (2) valid with restrictions
**31.08.2001** (91)

**Type** : Metabolism
**Remark** : Following incubation of o-nitroaniline with rabbit liver microsomes,4-amino-3-nitrophenol was cited as the major metabolite. This compound has the RNCAS 610-81-1. An internal data indicate an oral toxicity of 1100 mg/kg which is in good agreement with the value retained for ortho-nitroaniline.
**Source** : Rhodia Recherches Saint Fons
**Reliability** : (3) invalid
Very old study, no data available on the conditions of realisation of the study, no GLPs, but evaluated by BUA
**Flag** : non confidential, Risk Assessment
**31.08.2001** (92)

**Type** : other: LD50/QSAR
**Method** : The acute oral mammalian toxicity (LD50) of a diverse set of substitued

anilines was studied using a quantitative structure-activity relationship (QSAR). Feature selection was performed using least median squares to evaluate the fitness of descriptors chosen by an evolutionary optimization routine. Using this method, a five-descriptor model was found with reasonable training set and prediction set root mean square (rma) errors. Computational neural networks further improved the model, yielding a training set rms error of 0.238 log units and a prediction set error of 0.254 log units. Additionnally, a feature selection routine using computational neural networks to evaluate the fitness of subsets of descriptors chosen by the genetic algorithm was employed. This routine was able to exploit the non-linear nature of a CNS, resulting in a model with a training set rms error of 0.233 log units and a prediction set rms error of 0.238 log units.

**Result** : For 2-nitroaniline, the mouse oral LD50 found in RTECS database is 1070 mg/kg, the LD50 calculated from model II, neural network is 783 mg/kg, and the LD50 calculated from the model III is 530 mg/kg.

**Source** : Rhodia Recherches Saint Fons

**Reliability** : (3) invalid
Non validated model

**Flag** : non confidential

31.08.2001 (93)

## 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**

**8.2 FIRE GUIDANCE**

**8.3 EMERGENCY MEASURES**

**8.4 POSSIB. OF RENDERING SUBST. HARMLESS**

**8.5 WASTE MANAGEMENT**

**8.6 SIDE-EFFECTS DETECTION**

**8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER**

**8.8 REACTIVITY TOWARDS CONTAINER MATERIAL**

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