FOREWORD INTRODUCTION

O-PHTHALODINITRILE

CAS N°: 91-15-6

SIDS Initial Assessment Report
for
12th SIAM
(France, June 27-29, 2001)

Chemical Name: o-Phthalodinitrile

CAS No: 91-15-6

Sponsor Country: Japan and Germany

National SIDS Contact Point in Sponsor Country:

Mr. Koji Tomita, Ministry of Foreign Affairs, Japan

Mr. Ernst Goedecke, Bundesanstalt fur Arbeitsschutz und Arbeitsmedizin, Germany

HISTORY:

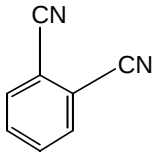
The original draft documents were prepared by BASF AG. Japanese and German Governments reviewed the documents after incorporation of Japanese testing results.

COMMENTS:

ICCA Initiative work lead by BASF AG., Germany

Deadline for circulation: March 31, 2001
Date of Circulation: April 15, 2001

SIDS INITIAL ASSESSMENT PROFILE

CAS Nr.	91-15-6
Chemical Name	o-Phthalodinitrile
Structural formula	

RECOMMENDATIONS

The chemical is currently of low priority for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

The chemical is acutely toxic by ingestion (rat oral LD_{50}: 85 mg/kg bw). The major effect is neurotoxicity. No mortality by inhalation occurred in rats exposed to saturated atmosphere with low dust formation for 8 hrs at 20 °C. It is considered as non-irritating to the skin and eyes. There are no available information on skin sensitization. In compliance with an OECD combined repeat dose and reproductive/ developmental toxicity screening test [TG 422], the chemical was given to male and female rats by gavage at doses of 0, 1, 6, 30 mg/kg bw /day for at least 42 days. Histopathological examination for the males of 30 mg/kg bw /day revealed centrilobular hypertrophy of hepatocytes in the liver, hyaline droplets in the proximal tubular epithelium, basophilic degeneration of the renal tubules and atrophy of the seminiferous tubules with cell debris in the tubules. In addition, the number of sperm in the epididymis significantly decreased in males of 30 mg/kg bw /day. No adverse effects were observed at 6 mg/kg bw/day. In a 13-week oral feeding study with rats conducted according to OECD TG 408 and US EPA guideline for neurotoxicity study, a reduced body weight gain which correlated with a reduced feed consumption was described. The substance caused an increase in motor activity, but no macroscopical or neurohistopathological correlations were found in the central and peripheral nervous system. Clouding of the lens was detected in eye examinations at the end of the study in both sexes in the high dose group and in some females in the intermediate dose group, an effect that was not evident after 4 weeks. Therefore the NOAEL for repeat dose toxicity was prescribed 3 mg/kg bw/day.

For gene mutations, the test results were uniformaly negative with and without an exogenous metabolic activation system in bacteria as well as mammalian cells, while the cytogenetic effect was judged to be positive in mammalian cells *in vitro* because of an increase of polyploid cells. However, this chemical did not show any cytogenetic effects in the well-planned *in vivo* micronucleus test. A weight of evidence suggests this chemical is not genotoxic *in vivo*.

In the above screening test [OECD TG 422], this chemical was given from 14 days before mating to 14 days after mating in males and from 14 days before mating to day 3 of lactation in females.

As all dams from the 30 mg/kg group died in late pregnancy, no data were obtained for after-delivery parameters. In the 1 mg/kg and 6 mg/kg groups, no changes due to administration of the chemical were observed. Therefore NOAEL for reproductive toxicity is considered to be 6 mg/kg/day in males and females. Any developmental toxicity including teratogenicity was not observed up to 6 mg/kg/day.

Available data (on carcinogenicity) were found to be invalid.

Old report indicates that irritation of skin and mucous membranes and cases of acute intoxication with dizziness, vomiting, unconsciousness, epileptiforme convulsions, and retrograde amnesia were described in workers after exposure to skin and by inhalation during handling. However a morbidity and a mortality study, and chromosome examinations in workers showed no abnormal findings.

Environment

This chemical has been tested in a limited number of aquatic species including fish, *Daphnia* and algae. For algae, acute toxicity values are 68 and 421 mg/L (72 h EbC_{50}) in *Selenastrum capricornutum* and *Scenedesmus subspicatus*, respectively. NOEC (72 h, biomass) of *Selenastrum* is 31.6 mg/L. For *Daphnia magna*, the acute toxicity values are 211 and 219 mg/L (48 h EC_{50} for immobilization), and the chronic value is 14 mg/L (21d NOEC for reproduction). For fish, only acute data are available; 96 h LC_{50} (*Oryzias latipes*) is 22.6 mg/L. PNEC of 0.14 mg/L for the aquatic organisms is calculated from 21 d-NOEC for *Daphnia* (14 mg/L) using an assessment factor of 100. This chemical is considered to be harmful to aquatic organisms.

Exposure

The production volume of this chemical in BASF AG Ludwigshafen, Germany was 1,000-5,000 t in 1999. The production volume is used as an intermediate (non disperse use) in chemical industry.

The substance is soluble in water (0.56g/l at 25 °C) and has no considerable potential for bio- and geoaccumulation. (BCF < 5.5, measured; log Kow=0.582 at 25°C) and turned out to be not readily biodegradable (OECD 301 E: 56-59 % after 4 days). However according to OECD 302 B the substance is inherently biodegradable with adapted inoculum (90-100 % after 12 days). In the atmosphere it is photodegraded very slowly (t1/2 = 350 d). Under environmental conditions no hydrolysis was observed. Distribution modeling using Mackay I indicates water to be the target compartment (99.4%) followed by air (0.6 %).

NATURE OF FURTHER WORK RECOMMENDED

O-Phthalodinitrile is an acute neurotoxicity hazard with effects seen at relatively low doses.

The chemical is a low priority for further work taking into consideration that it is manufactured at one site as a chemical intermediate. The SIAM was informed that exposure is adequately controlled at this site.

FULL SIDS SUMMARY

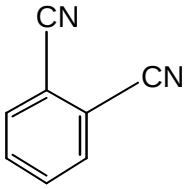
CAS NO: 91-15-6		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
2.1	Melting Point			141 - 142 °C
2.2	Boiling Point			304.5 °C (at 1013.3 hPa)
2.3	Density			1.24 g/cm^3 at 20 °C
2.4	Vapour Pressure		extrapolated	0.8 – 3.8 mPa @ 25°C
2.5	Partition Coefficient (Log Pow)			0.582 at 25 °C
2.6 A.	Water Solubility		OECD TG 105	0.56 g/L at 25 °C
B.	pH			
	pKa			
2.12	Oxidation: Reduction Potential			
ENVIRONMENTAL FATE AND PATHWAY				
3.1.1	Photodegradation		Calculated	$T_{1/2}$ = 350 days
3.1.2	Stability in Water			
3.2	Monitoring Data			
3.3	Transport and Distribution		Calculated (Level I Fugacity Model)	Air: 0.6 % Water: 99.4 % Soil: 0 %
3.5	Biodegradation		OECD 301E	56-59 % after 30 days
			OECD 302B	90-100 % after 12 days
3.7	Bioaccumulation		OECD 305 C	BCF < 5.5
ECOTOXICOLOGY				
4.1	Acute/Prolonged Toxicity to Fish	*Oryzias latipes*	OECD TG 203	LC_{50}(96hr)=22.6 mg/L
			Other	LC_{50}(24hr)=42.5 mg/L LC_{50}(48hr)=27 mg/L
			Other	LC_{50}(48hr)=29 mg/L
4.2	Acute Toxicity to Aquatic Invertebrates (*Daphnia*)	*Daphnia magna*	OECD TG 202	EC_{50}(48hr,Imm)=211 mg/L
			OECD TG 202	EC_{50}(24hr,Imm)=330 mg/L EC_{50}(48hr,Imm)=219 mg/L
4.3	Toxicity to Aquatic Plants e.g. Algae	*Selenastrum capricornutum*	OECD TG 201	EC_{50}(72hr,Bms)= 68mg/L NOEC(72hr,Bms)=31.6 mg/L
		Scenedesmus subspicatus	OECD TG 201	EC_{50}(72hr,Bms)= 421mg/L EC_{50}(96hr,Bms)= 415mg/L
4.5.2	Chronic Toxicity to Aquatic Invertebrates (*Daphnia*)	*Daphnia magna*	OECD TG 202	NOEC(21d,Rep)= 14 mg/L

4.6.1	Toxicity to Soil Dwelling Organisms			
4.6.2	Toxicity to Terrestrial Plants			
4.6.3	Toxicity to Other Non-Mammalian Terrestrial Species (Including Birds)			
TOXICOLOGY				
5.1.1	Acute Oral Toxicity	Rat	OECD TG 401	LD_{50} = 85 mg/kg b.w.
5.1.2	Acute Inhalation Toxicity	Rat	Inhalation Risk Test	No mortality within 8 hours in saturated vapour atmosphere (20°C) with low dust formation
5.1.3	Acute Dermal Toxicity			No study
5.2.1	Skin Irritation	Rabbit	Other	Not irritating
5.2.2	Eye Irritation	Rabbit	Other	No irritating
5.3	Skin Sensitisation			No study
5.4	Repeated Dose Toxicity	Rat	OECD TG 408	NOAEL = 3 mg/kg b.w./day
		Rat	OECD TG 422	NOAEL = 6 mg/kg b.w./day
5.5	Genetic Toxicity *in vitro*			
A.	Bacterial Test (Gene mutation)	*S. typhimurium, E. coli*	Japanese TG and OECD TG 471	- (With metabolic activation) - (Without metabolic activation)
B.	Non-Bacterial *in vitro* Test (Chromosomal aberrations)	CHL cells	Japanese TG and OECD TG 473	+ (With metabolic activation) + (Without metabolic activation)
5.6	Genetic Toxicity *in vivo*	Mouse	OECD TG 474	Negative
5.7	Carcinogenicity			No valid study
5.8	Toxicity to Reproduction	Rat	OECD TG 422	NOAEL Parental = 6 mg/kg/day (male) NOAEL Parental = 6 mg/kg/day (female)
5.9	Developmental Toxicity/ Teratogenicity	Rat	OECD TG 422	NOAEL maternal = 6 mg/kg b.w./day NOAEL F1 Offspring = 6 mg/kg b.w./day No teratogenicity
5.11	Experience with Human Exposure		studies with workers	No adverse effects in several reports of studies with workers
			case report	Cases of intoxication by accidental exposure

SIDS INITIAL ASSESSMENT REPORT

1. IDENTITY

Chemical Name:	o-Phthalodinitrile
Synonyms:	1,2-Benzenedicarbonitrile
	1,2-Dicyanobenzene
	1,2-Benzodinitrile
	o-Benzenedinitrile
CAS Number:	91-15-6
Empirical Formula:	$C_8H_4N_2$
Structure:	



General Substance Information (1)

Substance type:	organic
Physical status:	solid
Purity:	≥ 98 % w/w

Physical and chemical properties

o-Phthalodinitrile is a grayish-yellow, crystalline powder, which is soluble in water (0.56 g/L at 25 °C) and shows a low vapor pressure (VP= 0.8-3.8 mPa at 25 °C, this figure is extrapolated from measured vapor pressures). The log Kow was measured to 0.582 at 25 °C. Since the density of o-phthalodinitrile (1,24 g/cm^3 at 20°C) is slightly higher than that of water, sedimentation or stratification in surface waters in case of accidental losses cannot be excluded. (1,2,3,4,5)

2. GENERAL INFORMATION ON EXPOSURE

2.1. Production and import

In 1999, the substance was produced in Germany by one company in an amount of 1,000 - 5,000 t. The substance was not imported in 1999 into the European Union.

Quantity refers to world market.

2.2 Use Pattern

The main use for o-phthalodinitrile is as an intermediate in the chemical industry for the synthesis of other products, mainly phthalocyanine dyes. Minor amounts are used in the field of sintering metals.

2.3 Other information

None

3. ENVIRONMENT

3.1 Environmental Fate and Pathways

Distribution modeling using Mackay, Level I, indicates water to be the main environmental compartment (99.4 %) followed by air (0.6 %). The estimated value for the Henry's law constant of 0.05 $Pa \cdot m^3 \cdot mol^{-1}$ indicates that volatilization from an aquatic environment is not expected under environmental conditions. Under environmental conditions no hydrolysis of o-phthalodinitrile was observed. In the atmosphere o-phthalodinitrile is photodegraded very slowly (t1/2 350 d). Therefore long-range distribution via the atmosphere is possible. Under OECD 301 E test conditions, o-phthalodinitrile is not readily biodegradable (56-59 % DOC after 4 days: no further biodegradation occurred up to 30 days). The substance is inherently biodegradable with adapted inoculum. In bioaccumulation studies on fish BCFs of < 5.5 were found, indicating a low bioaccumulation potential. (6-11)

3.2 Toxicity to Aquatic Organisms

o-Phthalodinitrile has been tested in a limited number of aquatic species. Results are summarized in Table 1. Fish (*Oryzias latipes*) was the most sensitive among the organisms tested.

Table 1: Summary of effects of o-phthalodinitrile on aquatic organisms

Organism	Test duration	Result (mg/L)	Reference
Aquatic plants, e.g. algae			
Green alga (*Selenastrum capricornutum*)	72 h (s)	EC_{50} (Bms) = 68 (nc) NOEC(Bms) = 31.6 (nc)	Japan EA: 2000 (12)
(*Scenedesmus subspicatus*)	72 h (s) 96 h (s)	EC_{50} (Bms) = 421 (nc) EC_{50} (Bms) = 415 (nc)	BASF AG: 1987 (13)
Invertebrates			
Water flea (*Daphnia magna*)	48 h (cl, s)	EC_{50} (Imm) = 211 (nc)	Japan EA: 2000 (12)
	24 h (nr) 48 h (nr)	EC_{50} (Imm) = 330 (nc) EC_{50} (Imm) = 219 (nc)	BASF AG: 1987 (14)
	21 d (cl, ss)	NOEC(Rep) = 14 (nc)	Japan EA: 2000 (12)
Fish			
Medaka (*Oryzias latipes*)	96 h (ss)	LC_{50} = 22.6(nc)	Japan EA: 2000 (12)
	24 h (s) 48 h (s)	LC_{50} = 42.5 LC_{50} = 27	Tonogai *et al.*: 1982 (3)
	48 h (f)	LC_{50} = 29	Japan Chemical Industry Ecology-Toxicology and Information Center: 1992 (15)

cl = closed system, op = open system, f = flow through, s = static, ss = semi-static,
nr = not described for test type nc = nominal m = measured concentration
Bms = biomass Imm = immobilization Rep = reproduction

3.3 Toxicity to Terrestrial Organisms

There is no available information.

3.4 Other

There is no available information.

3.5 Initial Assessment for the Environment

The main environmental compartment to which o-phthalodinitrile is distributed if it is released to the environment, is considered to be water according to the distribution modeling using Mackay, level I. This chemical is not hydrolyzed and not readily biodegradable, but this chemical is inherently biodegradable with adapted inoculum. Bioaccumulation is not expected in aquatic organisms (BCF's < 5.5).

The lowest acute and chronic values are 22.6 mg/L (Medaka 96h LC_{50}) and 14 mg/L (*Daphnia* 21 d NOEC, reproduction), respectively. The predicted no effect concentration (PNEC) of 0.14 mg/L for the aquatic organisms is calculated from the 21 d-NOEC for *Daphnia* (reproduction) using an assessment factor of 100, because only two chronic data (*Daphnia* and *Selenastrum*) are available, however, not with the species that is most sensitive in acute studies (Medaka).

4. HUMAN HEALTH

4.1 Experience with Human Exposure

There were five reports on human effects of o-phthalodinitrile. One study (16,17) revealed that irritation of skin and mucous membranes, dizziness, nausea, vomiting, headache, loss of consciousness and epileptiforme convulsions were observed in production workers after absorption through the skin and following inhalation of dust. Symptoms appeared after a latency period of 0.5 – 48 hrs. One worker suffered vomiting and dizziness, a convulsive attack after falling down and – as a consequence - a fracture of the base of the skull with fatal result. Subsequent examination of the brain showed haematomas, contusion, and brain edema attributable to the fractur. However, this data was old and there was no information on an exposure level. In other four reports on a morbidity study on 81 workers (18), a mortality study on 83 production workers (19), studies of chromosomes from 20 production workers (20) and occupational medical examination of 1 production and 3 processing plants (21), no adverse effects were reported and there was also no information on an exposure level.

4.2 Effects on human health

4.2.1 Acute Toxicity

Seven studies using rats and mice were reported as shown in Table 2. MHW study was considered to be the most reliable because this study was well conducted under OECD TG 401. The details of this study are as follows.

SD rats (5/sex/dose) was administered by gavage at doses of 0 (vehicle), 30, 60, 120, 240, 480 mg/kg (22). Deaths occurred in the 60 mg/kg and more groups for both sexes. Convulsion and perioral smudge in dead animals of the 60 mg/kg and more groups were observed. Decrease in locomotor activity, prone position, vocalization, straub tail and cyanosis in dead animals of the 240 mg/kg and more groups were observed. No pathological changes were observed.

Table 2: Acute toxicity of o-phthalodinitrile in experimental animals

Route	Animals	Values	Type	References
Oral				
	Rat	85 mg/kg	LD_{50}	MHW, Japan: 2001 (22)
	Rat	125 mg/kg	LD_{50}	BASF: 1969 (23)
	Rat	125 mg/kg	LD_{50}	BASF: 1969 (24)
	Rat	30 mg/kg	LD_{50}	National Technical Information Service (25)
	Mouse	65.2 mg/kg	LD_{50}	Yoshikawa & Kawai: 1966 (26)
Inhalation				
	Rat*	Saturated vapour atmosphere**	LC_0	BASF: 1969 (23)
	Rat*	Saturated vapour atmosphere**	LC_0	BASF: 1969 (24)

* Inhalation hazard test

** No mortality occurred in rats exposed to saturated vapour atmosphere with low dust formation for 8 hrs at 20 °C.

Conclusions:

o-Phthalodinitrile is acutely toxic by ingestion (rat oral LD50: 85 mg/kg bw). The major effect is neurotoxicity. No mortality by inhalation occurred in rats exposed to saturated vapour atmosphere with low dust formation for 8 hrs at 20 °C.

4.2.2 Corrosiveness and Irritation

Application of 0.5 ml/animal of a 50 % aqueous emulsion to rabbits (surface area 2 x 2 cm) for 1 min up to 20 hours did not induce skin irritation (23,24).

The undiluted substance (0.1 ml bulk volume/animal) was not irritating to eyes in rabbits (23,24).

Conclusions:

This chemical is not irritating to skin and eyes in rabbits.

4.2.3 Skin sensitization

There were no available data.

4.2.4 Repeated Dose Toxicity

Three studies were carried out by oral administration (gavage or feeding) in exposure period ranging from 14 days to 13 weeks (Table 3). Common toxic effects among three reports were a reduced body weight gain, probably due to reduced food intakes. In 14-day study (27), conducted as dose finding study for the 13 week study, rats were given at 5, 15, 45 mg/kg/day for 14 days by

dietary incorporation. Decrease in body weight gain with reduced food intakes was only induced and NOAEL was considered 15 mg/kg/day. MHLW study (22) and 13-week study (28) were considered to be the most reliable because the studies were well conducted and reported. The details of these studies are as follows.

In MHLW repeated oral dose toxicity test in compliance with OECD TG 422, SD (Crj: CD) rats received o-phthalodinitrile by gavage at doses of 0, 1, 6 and 30 mg/kg/day (22). Males were dosed for 44 days and females were dosed from 14 days before mating, throughout pregnancy to day 3 of lactation.

All females of the 30 mg/kg group died on days 19-23 of gestation, some of them developing convulsions. For these dead females, blood biochemical examination and organ weight measurement were not conducted. Body weight gain was suppressed and decrease in food consumption was observed in males and females in the 30 mg/kg group. In blood biochemical examination, increases of total cholesterol and total protein in 30 mg/kg males, and increase of total protein (5.9 % increase of control) in 6 mg/kg females were observed. Increased absolute and relative weight of the liver, increase relative weight of kidneys and testes and decreased absolute and relative weight of the epididymis were noted in male of 30 mg/kg group. Histopathological examination for the males of 30 mg/kg revealed centrilobular hypertrophy of hepatocytes in the liver, hyaline droplets in the proximal tubular epithelium, basophilic degeneration of the renal tubules, and atrophy of the seminiferous tubules with cell debris in the tubules of epididymis. The number of sperm in the epididymis significantly decreased in males of 30 mg/kg/day. As the result of L.F.B. dyeing for the females which showed convulsion, the histological change as a cause of the death was not noticed. Renal change seems to be due to accumulation of the chemical complex with male rat specific protein, alpha-2u-globulin. As an increase of total protein in 6 mg/kg female was not considered as adverse effect, NOAEL for repeated dose toxicity is considered to be 6 mg/kg/day for both sexes.

The death with convulsions, which was observed on late gestation period in 30 mg/kg females, was not observed in males. Furthermore, this effect was not observed in both sexes in other repeated dose studies up to 45 mg/kg. Since this death with convulsion was observed at 60 mg/kg and more in acute toxicity study, it was suggested that the lethality in 30 mg/kg pregnant females was induced by increase in substantial maternal intake of test substance due to fetal growth during late pregnancy.

In a 13 week oral feeding study with rats according to the OECD guideline 408 and the US EPA guideline for neurotoxicity study, SD rats received gavage doses of 0, 3, 8 and 25 mg/kg in males, and 0, 3, 10 and 30 mg/kg/day in females (28). A thorough neurotoxicological study (FOB: Functional Observational Battery), specific neuropathological study and ophthalmological evaluations were carried out in addition to hematological, blood biochemical and histopathological examination.

A reduced body weight gain (body weights were 87 % and 80 % compared to controls for males and females, respectively at the end of the study), which correlated to a reduced feed consumption, was observed in both sexes of high dose group. Histopathological examination indicated kidney lesions (hyaline droplet formation in tubules) in males of 8 and 25 mg/kg/day groups but this change was considered to be a species-specific change as observed in MHW study. During FOB, the substance caused a significant increase in motor activity in males of the high dose group and in females of the high and intermediated dose group but no macroscopical or neurohistopathological correlations were found in the central and peripheral nervous system. In opthalmological evaluations, clouding of the lens (focal cataracts involving posterior cortex at the junction of suture

lines in 60 % of high dosed males and females and in 20 % of mid dose females) was detected at the end of the study in both sexes of the high dose group and in some females in the intermediate dose group, an effect that was not evident after 4 weeks. The cataracts were not extensive enough to decrease vision at the time of examination The NOAELs are considered to be 8 mg/kg/day in male and 3 mg/kg/day in female.

Table 3: Repeated oral dose toxicity of o-phthalodinitrile in rats

Route	Animal	Period	Doses	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Toxic effects	Reference
Feed	Rats	14 days	5 - 45 mg/kg	15	45	Decreased BW gain	BG Chemie: 1995 (27)
Gavage	Rats	44 days	1 - 30 mg/kg	6	30	Death with convulsion, decreased BW gain, liver and testis change	MHLW, Japan: 2001 (22)
Feed	Rats	13 weeks	3 - 30 mg/kg	3	10	Decreased BW gain, increase in motor activity, lens clouding	BG Chemie: 1995 (28)

Conclusions:

In three studies in rats by oral administration (gavage or feeding) for 14 days to 13 weeks, major effects were a reduced body weight gain, which correlated to a reduced feed consumption, death associated with convulsion and histopathological changes in kidney. In addition, some histopathological changes in liver and testes, increased locomotor activity during FOB and clouding of the lens in opthalmological evaluations were also observed. The NOAELs for repeated dose toxicity is considered to be 8 mg/kg/day in male and 3 mg/kg/day in female.

4.2.5 Genetic Toxicology

Two bacterial tests, two non-bacterial *in vitro* tests and one genetic *in vivo* test were reported. The summary was shown in the following Table 4.

Table 4: Summary of genotoxicity studies on o-phthalodinitrile

Type of test	Test system	Dose	Result	Reference
Bacterial test				
Ames test *(reverse mutation)*	*S. typh.* (strains TA100, TA1535, TA98, TA1537) *E. coli* WP2 uvr A	Up to 5,000 ug/plate	**Negative** (+ & - MA*)	MHLW, Japan: 2001 (22)
Ames test *(reverse mutation)*	*S. typh.* (strains TA100, TA98, TA1537, TA1535)	Up to 3,000 ug/plate	**Negative** (+ & - MA)	BASF: 1978 (29)
Non-bacterial in vitro test				
Chromosomal aberration test	CHL/IU cells	Up to 1.3 mg/ml	**Positive** for polyploids (+ & - MA) **Negative** for structural aberration	MHLW, Japan: 2001 (22)
HGPRT assay	V79 cells	Up to 230 ug/ml	**Negative** (+ & - MA)	Laboratorium: 1987 (30)
Genetic in vivo test				
Micronucleus test	Mouse	Up to 20 mg/kg bw	**Negative**	Laboratorium: 1987 (31)

*MA: Metabolic activation

Bacterial test
MHW study was well conducted and reported under Japanese Guideline for Screening Mutagenicity Testing of Chemicals and OECD TG 471 (22). Results show negative in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 *uvr*A with or without an exogenous metabolic activation system.

Non-bacterial in vitro test
Chromosomal aberration test and HGPRT assay were reported as a non-bacterial *in vitro* test. Both studies were well conducted and reported. Details are as follows.

Chromosomal aberration test was conducted in cultured Chinese hamster lung (CHL/IU) cells by Japanese test guideline equivalent to OECD TG 473 (22). Structural chromosomal aberrations were not induced in 24h-continuous treatment and in short-term treatment. But polyploid cells were significantly increased in the 24h-continuous treatment (frequencies: 0.25 – 4.13 %) and the short-term treatment (frequencies: 1.13 – 9.50 without S9 mix and 5.14 – 26.13 % with S9 mix) with and without metabolic activation.

HGPRT assay was conducted in V79 cells by OECD TG 476 (30). The test substance, which was assessed in two independent experiments, did not enhance the mutation rate over the range of the negative controls without and with metabolic activation. The test substance was applied with the maximally soluble amount in the test medium.

Genetic in vivo test
Only one study was reported (31). This study was well conducted by OECD TG 474 and reported. Details of this study are as follows.

The test substance was administered to mice per os (gavage) twice separated by 24 hours (31). 5 males and 5 females per group were treated at doses of 2, 7, or 20 mg/kg b.w./treatment. The high dose was established based on the result of pre-experiment, in which 20 mg/kg (double administration) of the test substance was the highest non-lethal dose. The bioavailability of the test substance in blood was proven since there were substance-induced symptoms (e.g. reduction of spontaneous reaction, piloerection); since there is no barrier between blood and bone marrow it can be concluded that the substance reached bone marrow. The preparation time for the solvent control and the 20 mg/kg dose were 6, 24, 48 hours after the second of the two administrations. The sampling time for 2 and 7 mg/kg was 6 hours after the second administrations. Enhanced micronucleus rates were not found at any time point in any of the dose groups.

As for polyploidy, biological significance of *in vitro* euploidy is unclear at present. But it is considered that the induction of euploidy is one of indicators of aneuploid induction, which is closely related to congenital chromosomal aberration syndrome, reproductive toxicity and teratogenicity. In this case, a clear induction of polyploidy was given in chromosomal aberration test *in vitro* only, but the negative result of *in vivo* micronucleus test demonstrates that the genotoxic potential or risk would be low in higher organisms.

Conclusions:
Test results for gene mutations by o-phthalodinitrile were uniformly negative with and without an exogenous metabolic activation system in bacteria as well as mammalian cells, while the cytogenetic effect was judged to be positive in mammalian cells *in vitro* because of an increase of polyploid cells. However, this chemical did not show any cytogenetic effects in the well-planned *in vivo* micronucleus test. A weight of evidence suggests this chemical is not genotoxic *in vivo*.

4.2.6 Carcinogenicity

There is one Russian study reported in 1972 (32). Rats and mice were administered with test substance via oral, dermal or s.c. route over 2 years and some tumours were observed. However, this study was regarded as invalid because no data were reported on the frequency of occurrence of tumors in the control groups or on histrical controls, and on the causes of death.

4.2.7 Toxicity to Reproduction

The only available study is an OECD combined repeated dose and reproductive/ developmental toxicity screening test [OECD TG 422] (22; see 4.2.4). This study was well conducted and reported. Details of this study are as follows.

o-Phthalodinitrile was administered to SD (Crj: CD) rats by gavage at doses of 0, 1, 6 and 30 mg/kg from 14 days before mating to 14 days after mating in males and from 14 days before mating to day 3 of lactation in females (22).

The compound had no effects on reproductive parameters such as the estrous cycle, copulation index, fertility index, number of corpora lutea, number of implantations, or implantation index. As all dams from the 30 mg/kg group died in late pregnancy, no data were obtained for the delivery index, gestation index, gestation length, parturition state, and lactation behavior. For these parameters, no changes due to administration of the compound were observed in the 1 and 6 mg/kg groups. Atrophy of seminiferous tubules was observed with cell debris in the tubules and decreased number of sperm in epididymis at 30 mg/kg/day. Therefore NOAEL is considered to be 6 mg/kg/day for male and female reproductive toxicity.

Conclusions:
As all dams from the 30 mg/kg group died in late pregnancy, no data were obtained on after-delivery parameters. Testicular toxicity was observed at 30 mg/kg group. At this dose there was also systemic distinct toxicity, e.g. in liver and kidneys (details see 4.2.4). In the 1 and 6 mg/kg groups, no changes due to administration of the compound were observed. The NOAEL is considered to be 6 mg/kg/day for male and female reproductive toxicity.

4.2.8 Developmental Toxicity /Teratogenicity

The only available study is an OECD combined repeated dose and reproductive/ developmental toxicity screening test (22; see 4.2.4). This study was well conducted and reported according to OECD TG 422 and GLP although there is no sufficient evidence on teratogenicity because the prenatal examination was not scheduled. Details of this study are as follows.

o-Phthalodinitrile was administered to SD (Crj: CD) rats by gavage at doses of 0, 1, 6 and 30 mg/kg from 14 days before mating to 14 days after mating in males and from 14 days before mating to day 3 of lactation in females (22).

As all dams from the 30 mg/kg group died in late pregnancy, no data were obtained for developmental toxicity. In the 1 and 6 mg/kg groups, no changes attributable to the compound were observed in any parameters on the offsprings, including number of offspring or live offspring, live birth index, the sex ratio, body weight, viability index on day 4, and external and necropsy findings. The NOAEL is considered to be 6 mg/kg/day for developmental toxicity.

Conclusions:

In the 1 and 6 mg/kg group, no changes due to administration of the compound were observed. As all dams from the 30 mg/kg group died in late pregnancy, no data were obtained for developmental toxicity. Any developmental toxicity was not observed up to 6 mg/kg/day.

4.2.9 Other relevant information

A. Toxicodynamics, toxicokinetics

There is no available information.

B. Specific toxicities

Information on structurally related chemicals

m-Phthalodinitrile (626-17-5)
The oral LD_{50} was 711 mg/kg in rats (33). This chemical has no irritating effect on eyes of rabbits (34). In 28-day repeated dose toxicity test in rat, clinical change (mainly salivation) and hypertrophy of hepatocyte in both sexes, and hyaline droplets in proximal tubules of the kidney in male were observed. Therefore, NOAEL was considered to be 8 mg/kg/day (35). The results of bacterial test and chromosomal aberration test *in vitro* are negative (35).

p-Phthalodinitrile (623-26-7)
The oral LD_{50} was 2,000 mg/kg/day in rats (36). In a 28-day repeated dose toxicity test in rats, centrilobular hypertrophy of hepatocyte in liver, irregular shaped follicles in the thyroid gland and hyaline droplets deposition in tubular epithelium in kidney were observed only in male. NOAEL was considered to be 5 mg/kg/day (36). The results of bacterial test and chromosomal aberration test *in vitro* are negative (36).

There are several common toxicities among all phthalodinitrile isomers including low repeated dose NOAELs ranging 3 to 8 mg/kg/day, their major toxicities such as hepatotoxicity in both sexes and renal toxicity in males, and non-genotoxic potential. These results suggest all isomers might be distributed in similar rates and tissues, and act via a similar mechanism.

4.3 Initial Assessment for Human Health

There is no available information on toxicokinetics and metabolism of o-phthalodinitrile. The chemical is acutely toxic by ingestion (rat oral LD_{50}: 85 mg/kg bw). The major effect is neurotoxicity. No mortality by inhalation occurred in rats exposed to saturated vapour atmosphere with low dust formation for 8 hrs at 20 °C. This chemical is not irritating to skin and eyes in rabbits. There is no available information on skin sensitisation.

Two highly reliable studies for repeated dose toxicity were conducted. In compliance with an OECD combined repeated dose and reproductive/ developmental toxicity screening test [TG 422], o-phthalodinitrile was given to Crj: CD (SD) male and female rats by gavage at doses of 0, 1, 6, 30 mg/kg/day for at least 42 days. In 30 mg/kg/day, all females died, some of them developing convulsions on days 19-23 of gestation. Histopathological examination for the males of 30 mg/kg revealed centrilobular hypertrophy of hepatocytes in the liver, hyaline droplets in the proximal tubular epithelium, basophilic degeneration of the renal tubules and atrophy of the seminiferous tubules with cell debris in the tubules. In addition, the number of sperm in the epididymis significantly decreased in males of 30 mg/kg bw /day. Kidney change seems to be due to the accumulation of the male rat specific protein complex, alpha-2u-globulin. No adverse effects were

observed at 6 mg/kg bw/day. - In a 13-week oral feeding study conducted according to OECD TG 408 and US EPA guideline for neurotoxicity study, rats received gavage doses of 0, 3, 8 and 25 mg/kg in males, and 0, 3, 10 and 30 mg/kg in females and reduced body weight gain which correlated with a reduced feed consumption was described in the high-dose group. Histopathological examination indicated kidney lesions (hyaline droplet formation in tubules) in males of 8 and 25 mg/kg/day groups but this change was considered to be a species-specific change as observed in the above study. During FOB a significant increase in motor activity in the high dose group in males and in the high and intermediated dose group in females was observed. But no macroscopical or neurohistopathological correlations were found in the central and peripheral nervous system. Clouding of the lens was detected in eye examinations at the end of the study in both sexes in the high-dose group and in some females in the intermediate dose group. Therefore, the NOAEL for repeated dose toxicity was prescribed 3 mg/kg bw/day.

The test results for gene mutations by o-phthalodinitrile were uniformly negative with and without an exogenous metabolic activation system in bacteria as well as mammalian cells, while in a cytogenetic test in mammalian cells *in vitro* there was no induction of structural chromosome aberrations but an increase of polyploid cells. However, this chemical did not show any cytogenetic effects in the well-planned *in vivo* micronucleus test. A weight of evidence suggests this chemical is not genotoxic *in vivo*.

In the above screening test [OECD TG 422], o-phthalodinitrile was given from 14 days before mating to 14 days after mating in males and from 14 days before mating to day 3 of lactation in females. As all dams from the 30 mg/kg group died in late pregnancy, no data were obtained for after-delivery parameters. Testicular toxicity was observed at 30 mg/kg group together with distinct toxic effects in liver and kidney. In the 1 mg/kg and 6 mg/kg groups, no reproductive effects due to administration of the chemical were observed. Therefore, the NOAEL for reproductive toxicity is considered to be 6 mg/kg/day in males and females. Any developmental toxicity including teratogenicity was not observed up to 6 mg/kg/day.

There is one available data on carcinogenicity but this was regarded as invalid.

Old report indicates that irritation of skin and mucous membranes and cases of acute intoxication with dizziness, vomiting, unconsciousness, epileptiform convulsions, and retrograde amnesia were described in workers after exposure to skin and by inhalation during handling. However, a morbidity and a mortality study, and chromosome examinations in workers showed no abnormal findings.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Physical/chemical property, production, use and distribution

The production volume of this chemical in BASF AG Ludwigshafen was 1,000-5,000 t in 1999. The production volume is used as an intermediate (non disperse use) in chemical industry. The substance has no considerable potential for bio- and geoaccumulation. (BCF < 5.5, measured) and turned out to be not readily biodegradable (OECD 301 E: 56-59 % after 4 days and no further biodegradation up to 30 days). However OECD 302 B the substance is inherently biodegradable with adapted inoculum (90-100 % after 12 days). In the atmosphere it is photodegraded very slow. Under environmental conditions no hydrolysis was observed.

Enviroment
This chemical has been tested in a limited number of aquatic species including fish, Daphnia and algae. For algae, acute toxicity values are 68 and 421 mg/L (72 h EbC_{50}) in *Selenastrum capricornutum* and *Scenedesmus subspicatus*, respectively. NOEC (72 h, biomass) of *Selenastrum* is 31.6 mg/L. For *Daphnia magna*, the acute toxicity values are 211 and 219 mg/L (48 h EC_{50} for immobilization), and the chronic value is 14 mg/L (21d NOEC for reproduction). For fish, only acute data are available; 96 h LC_{50} (*Oryzias latipes*) is 22.6 mg/L.

PNEC of 0.14 mg/L for the aquatic organisms is calculated from 21 d-NOEC for *Daphnia* (14 mg/L) using an assessment factor of 100. This chemical is considered to be harmful to aquatic organisms.

Human health
Oral LD_{50} value is 85 mg/kg in rats. This chemical is not irritating to skin and eyes. Repeated dose studies show death in relatively high dose (30 mg/kg bw) as compared to acute toxicity (80 mg/kg bw) associated with convulsion and some histopathological changes in liver, kidney and testis. In addition, increased locomotor activity during FOB and clouding of the lens in opthalmological evaluations were observed. The NOAELs for repeated dose toxicity is considered to be 3 mg/kg/day. As for reproductive/developmental toxicity, since all dams from the 30 mg/kg group died in late pregnancy, no data were obtained for after-delivery parameters. Based on no reproductuve effects up to 6 mg/kg/day, NOAEL for reproductive toxicity is considered to be 6 mg/kg/day in males and females. Any developmental toxicity including teratogenicity was not observed up to 6 mg/kg/day. Genotoxic potential of this chemical was mostly negative in *in vitro* and *in vivo* studies, while in a mammalian *in vitro* test there was an increase in polyploid cells. However, a weight of evidence suggests this chemical is not genotoxic *in vivo*.

o-Phthalodinitrile is an acute neurotoxicity hazard with effects seen at relatively low doses. However, this chemical is a low priority of further work taking into consideration that it is manufactured at one site as a chemical intermediate. The SIAM was informed that exposure is adequately controlled at this site.

5.2 Recommendations

No recommendations.

REFERENCES

(1) BASF AG, Safety Data Sheet, 13-05-1998

(2) BASF AG, unpublished data (75/1418), 14.01.1987

(3) Tonogai Y. et al.: J. Toxicol. Sci. 7, 193-203 (1982)

(4) BASF AG, unpublished data (22-848), 18.11.1975

(5) BASF AG, unpublished data (UV 01.87), 01.09.1987

(6) Atkinson, R.: A structure-activity relationship for the estimation of rate constants for the gas-phase reactions of OH radicals with organic compounds. Int. J. Chem. Kin. 19, 799 - 828 (1987)

(7) Christen, H.R.: Organische Chemie. 6. Auflage, Verlag Salle und Sauerlaender (1985)

(8) BASF AG, department of ecology, unpublished calculation, 20.12.1998

(9) BASF AG, department of ecology, unpublished data (88/0662), 24.02.1989

(10) BASF AG, department of ecology, unpublished data (66691), 25.10.1976

(11) Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan, edited by Chemicals Inspection & Testing Institute Japan, published by Japan Chemical Industry Ecology-Toxicology & Information Center, October 1992

(12) Environment Agency Japan (2000)

(13) Japan Chemical Industry Ecology-Toxicology and Information Center October 1992

(14) BASF AG, department of ecology, unpublished data (0612/87), 30.07.1987

(15) BASF AG, department of ecology, unpublished data (0612/87), 20.07.1987

(16) Thiess A. M., Zbl. Arbeitsmed. 18, 303-312, (1968)

(17) Zeller H. et al., Zbl. Arbeitsmed. 19, 225-238, (1969)

(18) Kleinsorge H., et al., Zbl. Arbeitsmed. 29, 130-132, (1979)

(19) Frentzel-Beyme R., et al., Zbl. Arbeitsmed. 29, 121-127, (1979)

(20) Fleig I., Thiess A. M., Zbl. Arbeitsmed. 29, 127-129, (1979)

(21) BASF AG, Werkärztlicher Dienst, unveröffentlichte Mitteilung, (1995)

(22) Ministry of Health, Labour and Welfare: Japan (2001), Toxicity Testing Reports of Environmental Chemicals 8, 261-289.

(23) BASF AG, Department Toxicology, unpublished data, XVIII 308, 09.01.69

(24) BASF AG, Department Toxicology, unpublished data, XVIII 307, 09.01.69

(25) National Technical Information Service. (Springfield VA 22161) Formerly U.S. Clearinghouse for Scientific & Technical Information. OTS0540933.

(26) Yoshikawa H and Kawai K, Ind. Health, 4, 11 (1966)

(27) BG Chemie, Heidelberg, project No.: 97206, Research report of Bio-Research-Laboratories LTD, Canada, 03/10/1995, and cited in: o-Phalodinitril, Toxikologische Bewertung, BG Chemie No. 28, 06/95

(28) BG Chemie, Heidelberg, project No.: 83536, Research report of Bio-Research-Laboratories LTD, Canada, 03/10/1995, and cited in: o-Phalodinitril, Toxikologische Bewertung, BG Chemie No. 28, 06/95

(29) BASF AG, Department Toxicology, unpublished results, (77/586), 01-20-1978

(30) Laboratorium für Mutagenitätsprüfungen, TH Darmstadt, unpublished results, sponsored by BG Chemie, Report No. LMP 271A, May 25 1987

(31) Laboratorium für Mutagenitätsprüfungen, TH Darmstadt, unpublished results, sponsored by BG Chemie, Report No. LMP 271B, May 27 1987

(32) Pliss GB and Volfson NI, Vop. Onkot., 18, 81-86 (1972)

(33) Cheav SL et al. (1990) Ann. Pharm. Fr., 48, 23.

(34) Owen (1972) Project Report No. 725-242, Huntingdon Research Center

(35) Ministry of Health and Welfare: Japan (1996), Toxicity Testing Reports of Environmental Chemicals 4, 507-527.

(36) Ministry of Health and Welfare: Japan (1996), Toxicity Testing Reports of Environmental Chemicals 3, 355-381.