FOREWORD INTRODUCTION

INDIGO BLUE

3H-Indol-3-one, 2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro

CAS N°: 482-89-3

SIDS Initial Assessment Report

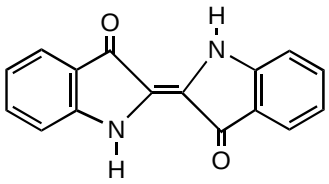
For

SIAM 2

Paris, France, 4-6 July 1994

1. Chemical Name:	3H-Indol-3-one, 2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro- (Indigo Blue)
2. CAS Number:	482-89-3
3. Sponsor Country:	Japan National SIDS Contact Point in Sponsor Country: Mr. Yasuhisa Kawamura, Ministry of Foreign Affairs, Japan
4. Shared Partnership with:	
5. Roles/Responsibilities of the Partners:	
• Name of industry sponsor /consortium	
• Process used	
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals Programme ?	As a high priority chemical for initial assessment, Indigo Blue was selected in the framework of OECD HPV Chemicals Programme. SIDS Dossier and Testing Plan were reviewed at a SIDS Review Meeting in 1993. At SIAM-2, the conclusion was approved with comments. Comments at SIAM-2: Rearrangement of the documents.
7. Review Process Prior to the SIAM:	
8. Quality check process:	
9. Date of Submission:	Date of Circulation: March 1994
10. Date of last Update:	
11. Comments:	

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	482-89-3
Chemical Name	3H-Indol-3-one, 2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro- (Indigo Blue)
Structural Formula	

CONCLUSIONS AND RECOMMENDATIONS

It is currently considered of low potential risk and low priority for further work.

SHORT SUMMARY WHICH SUPPORTS THE REASONS FOR THE CONCLUSIONS AND RECOMMENDATIONS

The production volume of Indigo Blue was ca. 1,200 tonnes/year in 1990 - 1992 in Japan. This chemical is used in dyeing industry as a direct dye or as an intermediate for the synthesis of other dyes. This chemical is considered as "not readily biodegradable".

PECs have been calculated based on several models considering its physico-chemical properties (e.g. molecular weight, water solubility, vapour pressure and partition coefficient). The worst estimated concentrations were 7.7×10^{-12} mg/l (air), 2.6×10^{-4} mg/l (water), 5.1×10^{-4} mg/kg (soil), 2.2×10^{-2} mg/kg (sediment).

For the environment, various NOEC and LC_{50} values were gained from test results; LC_{50} > 1000 mg/l (acute fish); EC_{50} = 250 mg/l (acute daphnia); EC_{50} = 6.5 mg/l (acute algae); NOEC = 0.78 mg/l (long-term daphnia reproduction). Therefore, the chemical is considered to be moderately toxic to daphnids and algae. The lowest chronic toxicity result, 21 d-NOEC (reproduction) of *Daphnia magna* (0.78 mg/l), was adopted for the calculation of PNEC, applying an assessment factor of 100. Thus the PNEC for the chemical is 0.0078 mg/l. Since the PEC is lower than the PNEC, environmental risk is presumably low.

Based on the physico-chemical properties, the level exposed indirectly through the environment was estimated to be 1.9×10^{-3} mg/man/day (i.e. 3.2×10^{-5} mg/kg/day). Also, the daily intake through drinking water is estimated to be 8.7×10^{-6} mg/kg/day and through fish is calculated to be 2.2×10^{-5} mg/kg/day. No data on occupational exposure are available. Neither monitoring data at work place nor data on consumer exposure have been reported.

Although the chemical showed no genotoxic effects in bacteria, a positive result was obtained from a chromosomal aberration test *in vitro*. However, in a micronucleus test *in vivo* that was performed to confirm the mutagenicity of the chemical, the result was negative.

In a 2-year feed study in rats, there were no serious effects related to the test substances up to the highest dose level (3% feed i.e. approx 1200 mg/kg/day). In a 3-generation reproductive toxicity study at doses of 5, 50, 150, or 500 mg/kg/day in rats, there were also no effects observed such as reproduction performance, maternal weight gain and fetal development. Therefore, the NOEL was 1,200 mg/kg/day for repeated dose toxicity as well as 500 mg/kg/day for reproductive toxicity.

For human health, estimated dose of low concern (EDLC) was calculated as 12 mg/kg/day and 5 mg/kg/day for repeated dose and reproductive toxicity, respectively, using a safety factor of 100. Daily intake of the chemical was estimated as 3.2×10^{-5} mg/kg/day from an exposure model. Also, the daily intake through drinking water is estimated to be 8.7×10^{-6} mg/kg/day and through fish is calculated to be 2.2×10^{-5} mg/kg/day. The EDLC is quite larger than the estimated human exposure, and the margin of safety is very large. Therefore, health risk through the environment, in general, is considered to be presumably low due to its use pattern and exposure situation.

In conclusion, no further testing is needed at present considering its toxicity and exposure levels.

NATURE OF FURTHER WORK RECOMMENDED

FULL SIDS SUMMARY

CAS NO: 482-89-3		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
2.1	Melting Point			300 °C
2.2	Boiling Point			390 °C (at 1013 hPa)
2.3	Density			No data available
2.4	Vapour Pressure		OECD TG 104	< 3.5 x 10^{-5} Pa at 100 °C
2.5	Partition Coefficient (Log Pow)		OECD TG 117	2.7 at 25 °C
2.6 A.	Water Solubility		OECD TG 105	0.99 mg/L at 25 °C
B.	pH			No data available.
	pKa			Not observed.
2.12	Oxidation: Reduction Potential			No data available.
ENVIRONMENTAL FATE AND PATHWAY				
3.1.1	Photodegradation		Estimation	$T_{1/2}$ = 0.112 y (direct photodegradation in water)
3.1.2	Stability in Water		OECD TG 111	Half-life: 622 days at pH 4 at 25 °C
3.2	Monitoring Data			No data available
3.3	Transport and Distribution		Calculated (MNSEM-147S)	In Air 7.7E-12 mg/L In Water 2.6E-4 mg/L In Soil 5.1E-4 mg/kg In Sediment 2.2E-2 mg/kg
3.5	Biodegradation		OECD TG 301C	Not readily biodegradable: 0 % (BOD) in 28 days.
3.6	Bioaccumulation	Carp	OECD TG 305C	BCF: 2.5 – 4.5
ECOTOXICOLOGY				
4.1	Acute/Prolonged Toxicity to Fish	*Oryzias latipes*	OECD TG 203	LC_{50} (24hr): > 1,000 mg/L LC_{50}(96hr): > 1,000 mg/L
4.2	Acute Toxicity to Aquatic Invertebrates (*Daphnia*)	*Daphnia magna*	OECD TG 202	EC_{50} (24hr): 250 mg/l
4.3	Toxicity to Aquatic Plants e.g. Algae	*Selenastrum capricornutum*	OECD TG 201	EC_{50} (72hr): 6.5 mg/l
4.5.2	Chronic Toxicity to Aquatic Invertebrates (*Daphnia*)	*Daphnia magna*	OECD TG 202	LC_{50} (21d, Mortality): 2.6 mg/l EC_{50} (21d, Reproduction): 1.6 mg/l NOEC (21d, Repro): 0.78 mg/l
4.6.1	Toxicity to Soil Dwelling Organisms			No data available.
4.6.2	Toxicity to Terrestrial Plants			No data available.
(4.6.3)	Toxicity to Other Non-Mammalian Terrestrial Species (Including Birds)			No data available
TOXICOLOGY				
5.1.1	Acute Oral Toxicity	Rat	Unknown	LD_{50} > 5,000 mg/kg
5.1.2	Acute Inhalation Toxicity			LC_{50}: 0.08 mg/L/4hr
5.1.3	Acute Dermal Toxicity			LD_{50} 2,000 mg/kg
5.4	Repeated Dose Toxicity	Rat	Unknown	NOEL = 1200 mg/kg/day
5.5	Genetic Toxicity In Vitro			
A.	Bacterial Test (Gene mutation)	*S. .typhimurium*	Unknown	Negative (With and without Metabolic activation)
B.	Non-Bacterial In Vitro Test (Chromosomal aberrations)	CHL cells	OECD TG 473 and Japanese Guideline	Positive (With and without metabolic activation)
5.6	Genetic Toxicity In Vivo	Mouse	Micronucleus Test	Negative
5.8	Toxicity to Reproduction	Rat	Unknown	NOEL Parental = 500 mg/kg/day NOEL F1 offspring = 500 mg/kg/day
5.9	Developmental Toxicity/	Rat	Unknown	NOEL Parental = 500 mg/kg/day

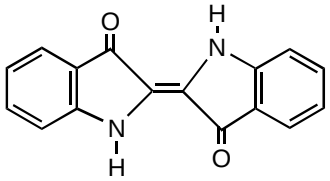
CAS NO: 482-89-3		SPECIES	PROTOCOL	RESULTS
	Teratogenicity			NOEL F1 offspring = 500 mg/kg/day
5.11	Experience with Human Exposure			

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 582-89-3
IUPAC Name: 3H-Indol-3-one, 2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene-1,2-dihydro-
Molecular Formula: $C_{16}H_{10}N_2O_2$
Structural Formula:



Synonyms: Indigo Blue

C.I. Vat Blue

D & C Blue No. 6

1.2 Purity/Impurities/Additives

Degree of Purity: 99 %

Major Impurities: unknown

Essential Additives: Sodium hydroxide

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value
Melting point	300 °C
Boiling point	390 °C
Vapour pressure	< 3.5 x 10^{-5} Pa at 100 °C
Water solubility	0.99 mg/l at 25 °C
Partition coefficient n-octanol/water (log value)	2.7

2 GENERAL INFORMATION ON EXPOSURE

Indigo Blue is a stable solid, and the production volume is ca. 1,200 tonnes/year in 1990 – 1992 in Japan. This chemical is used in dyeing industry as a direct dye or as an intermediate for the synthesis of other dyes. Indigo Blue seems to be released into water from its production sites after biological treatment. No specific monitoring data of the chemical are available. This chemical is stable in neutral, acidic or alkaline solutions, and is classified as "not readily biodegradable".

2.1 Environmental Exposure and Fate

2.1.1 Photodegradation

The half-life time of 0.112 years is estimated for the degradation of Indigo Blue in water by direct photodegradation. (MITI, Japan).

2.1.2 Stability in Water

The chemical is stable in water at pH 4, 7 and 9 (OECD TG 111). Half-life at pH 4 is 622 days at 25 °C

2.1.3 Biodegradation

If released into water, this substance is not readily biodegraded (MITI (I), corresponding to the OECD TG 301C: 0 % during 28 days based on BOD).

2.1.4 Bioaccumulation

BCF= 2.5 – 4.5 in carp (6 weeks at 25 °C) suggests that the potential for bioconcentration in aquatic organisms is low.

2.1.5 Estimates of environmental fate, pathway and concentration

Global situation

Method: MNSEM 147S

Input data:	Molecular weight:	262.27
	Water solubility:	7.50 [mg/l]
	Vapor pressure:	3.0E-07 [mmHg]
	Log Pow:	2.70

Results: Steady state mass and concentration

Air:	7.7E-12 [mg/l]
Water:	2.6E-04 [mg/l]
Soil:	5.1E-04 [mg/kg dry solid]
Sediment:	2.2E-02 [mg/kg dry solid]

Environmental exposure dose (Concentration in foods)

Inhalation of air:	1.4E-07 [mg/day]
Drinking water:	5.2E-04 [mg/day] (i.e. 8.6E-6 mg/kg/day)
Ingestion of fish:	1.3E-03 [mg/day] (i.e. 2.2E-6 mg/kg/day)
meat:	3.7E-09 [mg/day]
milk:	4.5E-09 [mg/day]
vegetation:	4.1E-05 [mg/day]
Total exposure dose:	1.9E-03 [mg/day] (i.e. 3.2E-5 mg/kg/day)

Comparison of calculated environmental concentration of Indigo Blue using several models.

Model	Air[mg/l]	Water[mg/l]	Soil[mg/kg]	Sediment[mg/kg]
MNSEM	7.7E-12	2.6E-04	5.1E-04	2.2E-02
CHEMCAN2	5.8E-12	2.6E-04	1.2E-04	3.2E-03
CHEMFRAN	4.9E-13	2.6E-04	7.5E-06	3.2E-03

2.2 Human Exposure

2.2.1 Occupational Exposure

No data on work place monitoring have been reported.

2.2.2 Consumer Exposure

No data on consumer exposure are available.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Acute Toxicity

The LD_{50} in an acute oral toxicity study in rats was reported as > 5.000 mg/kg.

LC_{50} and LD_{50} values in acute inhalation and dermal toxicity studies in rats and rabbit are 5.3 mg/L/4 hrs and > 2,000 mg/kg, respectively.

Indigo Blue is not irritating to rabbit skin and eyes, and not sensitizing to humans.

3.1.2 Repeated Dose Toxicity

There is 2 year oral repeated dose toxicity study in rats of Indigo Blue (Feber, K,H. 1987). Male and female F344/N rats were orally administered (feeding) at doses of 0, 0.25, 1.0 or 3.0% (0, 100, 400, 1,200 mg/kg/day) for 2 years. Appearance and behavior of the test rats were generally comparable to those of the controls. At the 3% level, food consumption was significant lower than in the controls for the first six months but comparable to controls during the remainder of the study. No significant difference on survival rates was noted. Significantly lower hematocrit and hemoglobin values in comparison with controls were obtained at all time intervals except 24 months for the males fed 3%. No such effect was noted in the females. Bilirubinuria was observed at 24 months in male and female at 1% and females at 3%. The test animals sacrificed at 52 weeks did not present any gross changes in the organs or viscera attributable to the test material. Autopsies performed on the animals which died during the second year of the study did not reveal any consistent gross changes. Microscopic findings at two years revealed no compound related effects on the kidneys or other tissues in either sex. The study appeared to demonstrate that after a period of adjustment to the higher dosage levels, the rats were able to tolerate up to of this substance in their diets without serious effects. NOEL for repeated dose toxicity is considered to be 1,200 mg/kg/day.

3.1.3 Mutagenicity

In vitro Studies

Bacterial test

In the reverse gene mutation assay, Indigo Blue showed negative results in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 with or without a metabolic activation system (Muzzall, J.M. & Cook, WL., 1979).

Non-bacterial test

A chromosomal aberration test in line with Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Test Guideline 473 was conducted using cultured Chinese Hamster lung (CHL/IU) cells. This study was well controlled and regarded as a key study. The maximum concentration of the chemical was used within no apparent cytotoxic effect in continuous treatment. In short term treatment, it was set to 2.5 mg/ml. Positive results were obtained with and without metabolic activation (MHW, 1994).

In vivo Studies

In a micronucleus test in mice at concentrations of 0, 0.1, 0.5, 1.0, 2.0 mg/kg, Indigo Blue showed negative results.

3.1.4 Toxicity for Reproduction

In a three-generation reproductive toxicity study at doses of 5, 50, 150 or 500 mg/kg/day in rats (Harlan-Wistar), there were also no effects observed such as reproduction performance, maternal weight gain and fetal development. Therefore, the NOEL is considered to be 500 mg/kg/day for reproductive toxicity. Also, an oral developmental toxicity study in CD rat at doses of 0, 50, 160, 500 mg/kg/day administered from day 6 through day 15 of gestation was carried out. On the basis of number of viable and dead fetuses, resorption site, mean fetal weight, sex distribution, mean litter size, frequency of anomalies or weight gain of pregnant females, Indigo Blue was without effect on reproduction performance, maternal weight gain and fetal development. The NOEL is considered to be 500 mg/kg/day for reproductive toxicity.

3.2 Initial Assessment for Human Health

Although the chemical showed no genotoxic effects in bacteria, positive result was obtained from chromosomal aberration test *in vitro*. However, in a micronucleus test *in vivo* that was performed to confirm the mutagenicity of the chemical, the results were negative.

In a 2-year feed study in rat, there were no serious effects related to the test substances up to the highest dose level (3% feed i.e. approx. 1200 mg/kg/day). In a 3 generation reproductive toxicity study a doses of 5, 50, 150,or 500 mg/kg/day in rat, there were also no effects observed such as reproduction performance, maternal weight gain and fetal development. Therefore, the NOEL was 1,200 mg/kg/day for repeated dose toxicity as well as 500 mg/kg/day for reproductive toxicity. Also, an oral developmental toxicity study in CD rat at doses of 0, 50, 160, 500 mg/kg/day administered from day 6 through day 15 of gestation was carried out. Indigo Blue showed no effects on reproduction performance, maternal weight gain and fetal development. the NOEL is considered to be 500 mg/kg/day for developmental toxicity.

For human health, daily intake of the chemical was estimated as 1.9×10^{-3} mg/man/day (i.e. 3.2×10^{-5} mg/kg/day) from an exposure model. Also, the daily intake through drinking water is estimated as 8.7×10^{-6} mg/kg/day and through fish is calculated as 2.2×10^{-5} mg/kg/day. The margin of safety is very large. Therefore, health risk through the environment, in general, is considered to be presumably low due to its use pattern and exposure situation.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Indigo Blue has been tested in a limited number of aquatic species (*Selenastrum capricornutum*, *Daphnia magna* and *Oryzias latipes*), under OECD test guidelines [OECD TG 201, 202 and 203]. Acute and chronic toxicity data to test organisms for the chemical are summarized in Table 2. No other ecotoxicological data are available. Various NOEC and LC_{50} values were gained from above tests; 96h LC_{50} > 1,000 mg/l (acute fish); 24h EC_{50} = 250 mg/l (acute daphnia); 72h EC_{50} = 6.5 mg/l (acute algae, biomass method); 72h NOEC = 3.1 mg/l (algae, biomass method); NOEC = 0.78 mg/l (long-term daphnia reproduction). These toxicities except the NOEC for daphnid reproduction were above the water solubility limit (0.99mg/l) of this chemicals, and it should be noted that the vehicle concentration of the control was as high as 1000 mg/l (DMSO:HCO40=9:1) in the daphnid reproduction test from which the lowest NOEC was driven. Therefore, the chemical is considered to be moderately toxic to daphnids and algae and non-toxic to fish. The lowest chronic toxicity result was the 21 d-NOEC (reproduction) for Daphnia magna (0.78 mg/l). An assessment factor of 100 is applied. Thus the PNEC of Indigo Blue is 0.0078 mg/l. Since the PEC is lower than the PNEC, the environmental risk is presumably low.

Table 2. Acute and chronic toxicity data of Indigo Blue to aquatic organisms.

Species	Endpoint[*1]	Conc. (mg/L)	Reference
Selenastrum capricornutum (algae)	Biomass: EC_{50} (72h) NOEC	6.5 mg/L 3.1 mg/L	EA, Japan. (1992)
Daphnia magna (water flea)	Imm.: EC_{50} (24h) Imm: EC_{50} (21d) Rep: EC_{50} (21d) NOEC (21d)	250 mg/L 2.6 mg/L 1.6 mg/L 0.78 mg/L	
Oryzias latipes (fish, Medaka)	Mor: LC_{50}(24h) Mor: LC_{50}(72h) Mor:LC_{50}(96h)	> 1,000 mg/L > 1,000 mg/L > 1,000 mg/L	

Notes: [*1] Mor; mortality, Rep; reproduction. Imm; Immobilisation

4.2 Initial Assessment for the Environment

For the environment, various NOEC and LC_{50} values were gained from test results; LC_{50} > 1000 mg/l (acute fish); EC_{50} = 250 mg/l (acute daphnia); EC_{50} = 6.5 mg/l (acute algae, biomass); 72h NOEC = 3.1 mg/l (algae, biomass method); NOEC = 0.78 mg/l (long-term daphnia reproduction). Therefore, the chemical is considered to be moderately toxic to daphnids and algae. The lowest chronic toxicity result is the 21 d-NOEC (reproduction) for Daphnia magna (0.78 mg/l). An assessment factor of 100 is applied. Thus the PNEC of the chemical is 0.0078 mg/l. Since the PEC is lower than the PNEC, the environmental risk is presumably low.

5 RECOMMENDATIONS

In conclusion, no further testing is needed at present considering its toxicity and exposure levels.

The chemical is considered of low potential risk and low priority for further work.

6 REFERENCES

EA, Japan (1992) "Investigation on the Ecotoxicological Effects of OECD High Production Volume Chemicals", Office of Health Studies, Environmental Health Department, Environment Agency, Japan (HPV/SIDS Test conducted by EA, Japan)

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Ferber, K.H. (1987) J. Environ. Pathol. Toxicol. Oncol., 7(4), 73-84 (US/FDA data).

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Lyman, W. J., W. F. Reehl and D. H. Rosenblatt (1981) "Handbook of Chemical Property Estimation Method", McGraw Hill Book Co.

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

SIDS DOSSIER

3H-Indol-3-one, 2-(1,3-dihydro-
3-oxo-2H-indol-2-ylidene)-
1,2-dihydro-

(Indigo Blue)

CAS No. 482-89-3

Sponsor country: Japan

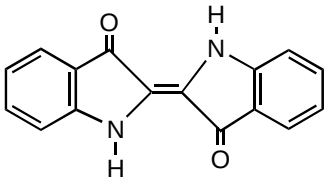
S I D S P R O F I L E

1.01 A.	**CAS No.**	482-89-3
1.01 C.	**CHEMICAL NAME (OECD Name)**	3H-Indol-3-one, 2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro- (Indigo Blue)
1.01 D.	**CAS DESCRIPTOR**	Not applicable
1.01 G.	**STRUCTURAL FORMULA**	$C_{16}H_{10}N_2O_2$
	OTHER CHEMICAL IDENTITY INFORMATION	
1.5	**QUANTITY**	In Japan 1,200 tonnes in 1990 - 1993.
1.7	**USE PATTERN**	(a) The chemical is used in dyeing industry for direct colorant or intermediate of dyes.
1.9	**SOURCES AND LEVELS OF EXPOSURE**	1. Amount released from production site to water is unknown. Waste gas is treated by bag filter, and then released. Option for disposal is incineration in Japan. 2. Amount released to air from production site is < 6.7 g/h. (estimation) 3. Detailed Information on consumer exposure is not available.
ISSUES FOR DISCUSSION (IDENTIFY, IF ANY)		

S I D S S U M M A R Y

CAS NO: 482-89-3		Information	OECD Study	GLP	Other Study	Estimation Method	Acceptable	SIDS Testing Required
STUDY		**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**
PHYSICAL-CHEMICAL DATA								
2.1	Melting Point	Y	N	N	Y	N	Y	N
2.2	Boiling Point	Y	N	N	Y	N	Y	N
2.3	Density	Y	N	N	Y	N	Y	N
2.4	Vapour Pressure	N						Y
2.5	Partition Coefficient	N						Y
2.6	Water Solubility	N						Y
	pH and pKa values	N						N
OTHER P/C STUDIES RECEIVED								
ENVIRONMENTAL FATE and PATHWAY								
3.1.1	Photodegradation	N						Y
3.1.2	Stability in water	N						Y
3.2	Monitoring data	N						N
3.3	Transport and Distribution	N						N
3.5	Biodegradation	N						Y
3.6	Bioaccumulation	Y	Y	Y	N	N	Y	N
OTHER ENV FATE STUDIES RECEIVED								
ECOTOXICITY								
4.1	Acute toxicity to Fish	N						Y
4.2	Acute toxicity to Daphnia	N						Y
4.3	Toxicity to Algae	N						Y
4.5.2	Chronic toxicity to Daphnia	N						Y
4.6.1	Toxicity to Soil dwelling organisms	N						N
4.6.2	Toxicity to Terrestrial plants	N						N
4.6.3	Toxicity to Birds	N						N
OTHER ECOTOXICITY STUDIES RECEIVED								
TOXICITY								
5.1.1	Acute Oral	Y	N	N	Y	N	Y	N
5.1.2	Acute Inhalation	Y	N	N	Y	N	Y	N
5.1.3	Acute Dermal	Y	N	N	Y	N	Y	N
5.4	Repeated Dose	Y	N	N	Y	N	Y	N
5.5	Genetic Toxicity *in vitro*							
	. Gene mutation	Y	N	N	Y	N	Y	N
	. Chromosomal aberration	N						Y
5.6	Genetic Toxicity *in vivo*	Y	N	N	Y	N	Y	N
5.8	Reproduction Toxicity	Y	N	N	Y	N	Y	N
5.9	Development / Teratogenicity	Y	N	N	Y	N	Y	N
5.11	Human experience	N						N
OTHER TOXICITY STUDIES RECEIVED								

1.01 SUBSTANCE INFORMATION

A.	**CAS-Number**	482-89-3
B.	**Name** *(IUPAC name)*	3H-Indol-3-one, 2-(1,3-dihydro-3-oxo-2Hindol-2-ylidene)-1,2-dihydro- (Indigo Blue)
C.	**Name** *(OECD name)*	3H-Indol-3-one, 2-(1,3-dihydro-3-oxo-2Hindol-2-ylidene)-1,2-dihydro-
D.	**CAS Descriptor**	Not applicable
E.	**EINECS-Number**	207-586-9
F.	**Molecular Formula**	$C_{16}H_{10}N_2O_2$
G.	**Structural Formula**	
H.	**Substance Group**	Not applicable
I.	**Substance Remark**	
J.	**Molecular Weight**	262.27

1.02 OECD INFORMATION

A.	**Sponsor Country:**	Japan
B.	**Lead Organisation:**	
	Name of Lead Organisation:	Ministry of Health and Welfare (MHW) Ministry of International Trade and Industry (MITI) Environment Agency (EA)
	Contact person:	Mr. Yasuhisa Kawamura Director Second International Organization Bureau Ministry of Foreign Affairs
	Address:	2-2-1 Kasumigaseki, Chiyoda-ku Tokyo 100, Japan TEL 81-3-3581-0018 FAX 81-3-3503-3136
C.	**Name of responder**	Same as above contact person

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance

element **[ ]**; inorganic **[ ]**; natural substance **[ ]**;

organic**[X]**; organometallic **[ ]**; petroleum product **[ ]**

B. **Physical State**

gaseous **[ ]**; liquid **[ ]**; solid **[X]**

C. **Purity** 99 %

1.2 **SYNONYMS** Indigo Blue,
C.I. Vat Blue
D & C Blue No.6

1.3 **IMPURITIES** Unknown

1.4 **ADDITIVES** Sodium hydroxide (6 %)

1.5 **QUANTITY**

Location	Production (tonnes)	Data
Japan	1,200	1990-1993

Export (tonnes)	1993	1992	1991
	750	790	760

Country: Spain, Italy, Belgium, Indonesia, Hong Kong, Thailand, Formosa

Reference: MITI, Japan

1.6 **LABELLING AND CLASSIFICATION**

Labelling None

Classification None

1.7 **USE PATTERN**

A. **General**

Type of Use:	**Category:**
main industry use	Dyeing industry

Remarks: None

Reference: MITI, Japan

B. **Uses in Consumer Products** None

Function	Amount present	Physical state
Clothes	Fiber	

Reference: MITI, Japan

1.8 **OCCUPATIONAL EXPOSURE LIMIT VALUE**

Source	Number of workers exposed	Frequency & duration of exposure	Emission data	Date
Packing	8	4h/day	< 10 mg/m^3	1990

Reference: MITI, Japan

1.9 SOURCES OF EXPOSURE

Source: Media of release: Air from a production site
Quantities per media: < 6.7 g/h
Remarks: Waste gas is treated by bag filter, and released.
Reference: MITI, Japan

1.10 ADDITIONAL REMARKS

A. Options for disposal Incineration

Reference: MITI, Japan

B. Other remarks None

2.1 MELTING POINT

Value:	300 °C
Decomposition:	Yes **[ ]** No **[ ]** Ambiguous **[ ]**
Sublimation:	Yes **[X]** No **[X]** Ambiguous **[ ]**
Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	None
Reference:	Farber, K.H. (1987)

2.2 BOILING POINT

Value:	390 °C
Pressure:	at 1013 hPa
Decomposition:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Method:	Unknown
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	None
Reference:	Merck Index (1982)

2.3 DENSITY

No studies located

2.4 VAPOUR PRESSURE

Value:	< 3.5 x 10^{-5} Pa
Temperature:	100 °C
Method:	calculated **[ ]**; measured [X] OECD Test Guideline 104
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Remarks:	
Reference:	MITI, Japan (1993)

2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

Log Pow:	2.7
Temperature:	25 °C
Method:	calculated **[ ]**; measured **[**X**]** OECD Test Guideline 117
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Remarks:	None
Reference:	MITI, Japan (1993)

2.6 WATER SOLUBILITY

A. Solubility

Value:	0.99 mg/l
Temperature:	25°C
Description:	Miscible**[ ]**; Of very high solubility **[ ]**; Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble **[ ]**; Of low solubility **[ ]**; Of very low solubility **[X]**; Not soluble **[ ]**
Method:	OECD Test Guideline
GLP:	Yes **[X]** No **[ ]** ? **[ ]**

Remarks:
Reference: MITI, Japan (1993)

B. pH Value, pKa Value

Not applicable

2.7 FLASH POINT

No studies located

2.8 AUTO FLAMMABILITY

No studies located

2.9 FLAMMABILITY

No studies located

2.10 EXPLOSIVE PROPERTIES

No studies located

2.11 OXIDIZING PROPERTIES

No studies located

2.12 OXIDATION: REDUCTION POTENTIAL

No studies located

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

No studies located

B. Other data

None

3.1 STABILITY

3.1.1 PHOTODEGRADATION

Type:	Air **[ ]**; Water **[X]**; Soil **[ ]**; Other **[ ]**	
Light source:	Sun light **[X]**; Xenon lamp **[ ]**; Other **[ ]**	
Light spectrum:		
Relative intensity:		
Spectrum of substance:	epsilon = 18200 at 300 nm epsilon = 17100 at 600 nm	
Concentration of Substance:		
Estimated parameter for calculation:		
	Quantum yield	0.0001
	Concentration	5×10^{-5} M
	Depth of water body	500 cm
	Conversion rate	6.023×10^{20}
Results:	Degradation rate	9.78×10^{-12}mol/l/s
	Half life	0.112 years
Reference	Lyman, W. J. et al. (1981)	

3.1.2 STABILITY IN WATER

Type:	Abiotic (hydrolysis) **[X]**; biotic (sediment)**[ ]**
Half life:	622 days at pH 4 at 25 °C
Method:	OECD Test Guideline 111
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Remarks:	None
Reference:	MITI, Japan (1993)

3.1.3 STABILITY IN SOIL

No studies located

3.2 MONITORING DATA (ENVIRONMENT)

No studies located

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

3.3.1 TRANSPORT

No studies located

3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota **[ ]**; Air-biota-sediment-soil-water **[ ]**; Soil-biota **[ ]**; Water-air **[ ]**; Water-biota **[ ]**; Water-soil **[ ]**; Other **[X]** (Air-soil-water-sediment)

Method: Fugacity level I **[ ]**; Fugacity level II [ ]; Fugacity level III **[X]**; Fugacity level IV **[ ]**; Other(calculation) **[ ]**;Other(measurement) **[ ]**

Results: Steady state mass and concentration calculated using MNSEM 147S

Air: 7.7E-12 [mg/l]

Water:	2.6E-04 [mg/l]
Soil:	5.1E-04 [mg/kg dry solid]
Sediment:	2.2E-02 [mg/kg dry solid]

Exposure dose

Inhalation of air:	1.4E-07 [mg/day]
Drinking water:	5.2E-04 [mg/day]
Ingestion of fish:	1.3E-03 [mg/day]
meat:	3.7E-09 [mg/day]
milk:	4.5E-09 [mg/day]
vegetation:	4.1E-05 [mg/day]
Total exposure dose:	1.9E-03 [mg/day]

Remarks: Input data:

Molecular weight:	262.27
Water solubility:	7.50 [mg/l]
Vapor pressure:	3.0E-07 [mmHg]
Log Pow:	2.70

MNSEM 147S is a slightly revised version of MNSEM 145I.
- addition of air particle compartment to air phase
- execution of calculation on a spreadsheet program

Comparison of calculated environmental concentration using several methods (Japanese environmental conditions are applied to the calculations.)

Model	Air[mg/l]	Water[mg/l]	Soil[mg/kg]	Sediment[mg/kg]
MNSEM	7.7E-12	2.6E-04	5.1E-04	2.2E-02
CHEMCAN2	5.8E-12	2.6E-04	1.2E-04	3.2E-03
CHEMFRAN	4.9E-13	2.6E-04	7.5E-06	3.2E-03

Reference: EA and MITI, Japan (1993)

3.4 IDENTIFICATION OF MAIN MODE OF DEGRADABILITY IN ACTUAL USE

No studies located

3.5 BIODEGRADATION

Type:	aerobic **[X]**; anaerobic **[ ]**
Inoculum:	adapted **[ ]**; non-adapted **[X]**; activated sludge, 30 mg/l as suspended solid
Concentration of the chemical:	related to COD **[ ]**; DOC **[ ]**; Test substance **[X]**;
Medium:	water **[ ];** water-sediment **[ ];** soil **[ ];** sewage treatment [ ] others **[X]** (Japanese standard activated sludge)
Degradation:	Degree of degradation after 28 days 0 % from BOD
Results:	Readily biodeg. **[ ];** Inherently biodeg. **[ ]**; under test condition no biodegradation observed **[X]**, Other **[ ]**
Method:	OECD Test Guideline 301C
GLP:	Yes **[X]** No **[ ]** ? **[ ]**

Remarks: None
Reference: MITI, Japan (1992)

3.6 BOD_5,COD OR RATIO BOD_5/COD

No studies located

3.7 BIOACCUMULATION

Species:	Carp
Exposure period:	6 weeks
Temperature:	25 °C
Concentration:	(1) 0.4 mg/l (2) 0.04 mg/l
BCF:	(1) 4.5 (2) < 2.5
Elimination:	Yes **[ ]** No **[ ]** ? **[ ]**
Method:	OECD Test Guideline 305C
Type of test:	calculated; **[X]** measured **[ ]** static **[ ]**; semi-static **[ ]**; flow-through **[X]**; other **[ ]**
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Remarks:	None
Reference:	MITI, Japan (1992)

3.8 ADDITIONAL REMARKS None

A. Sewage treatment

B. Other information

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type of test:	static **[ ]**; semi-static **[X]**; flow-through **[ ]**; other **[ ]**
	open-system **[X]**; closed-system **[ ]**
Species:	*Oryzias latipes*
Exposure period:	96 hr
Results:	LC_{50} (24h) = > 1000 mg/l
	LC_{50} (48h) = > 1000 mg/l
	LC_{50} (72h) = > 1000 mg/l
	LC_{50} (96h) = > 1000 mg/l
	NOEC =
	LOEC =
Analytical monitoring:	Yes **[ ]** No **[X]** ? **[ ]**
Method:	OECD Test Guideline 203 (1981)
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Test substance:	2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-Indol-3-one, purity = 96%
Remarks:	A group of 10 Oryzias latipes were exposed to 5 nominal Concentrations (100-1000 mg/l), control of DMSO:HCO-40 = 9:1 (100 mg/l) and laboratory water control.
Reference:	EA, Japan (1992)

(b)

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]**;
	open-system **[ ]** closed-system **[ ]**
Species:	*Oryzias Latipes* (Medaka)
Exposure period:	
Results:	LC_{50}(24h) = 63.0 mg/l
	LC_{50}(48h) = 42.0 mg/l
	LC_{50}(72h) =
	LC_{50}(96h) =
	LC_{50}(7d) =
	NOEC =
	LOEC =
Analytical monitoring:	Yes **[ ]** No **[ ]** ? **[X]**
Method:	
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	
Reference:	Tonogai, Y. et al. (1982)

(c)

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**;
	other **[ ]**;
	open-system **[ ]** closed-system **[ ]**
Species:	Goldorfe
Exposure period:	
Results:	LC_{50}(24h) =
	LC_{50}(48h) =
	LC_{50}(72h) =
	LC_{50}(96h) = >10000 mg/l
	LC_{0} (48h) =
	NOEC =
	LOEC =
Analytical monitoring:	Yes **[ ]** No **[ ]** ? **[X]**
Method:	

GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance:
Remarks:
Reference: Company data (Germany)
BASF AG (1989), Abteilung Toxicologie, unpublished report (88/398), 25/01/89

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

A. Daphnia

Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]**;
open-system **[**X**]**; closed-system **[ ]**
Species: *Daphnia magna*
Exposure period: 24 hrs
Results: $EC_{50\ (24h)}$ = 250 mg/l
$EC_{50\ (48h)}$ =
NOEC =
LOEC =
Analytical monitoring: Yes **[ ]** No **[X]** ? **[ ]**
Method: OECD Test Guideline 202 (1984)
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Remarks: 20 daphnids (4 replicates; 5 organisms per replicate) were exposed to 5 nominal concentrations (100-1000 mg/l), control of DMSO: HCO-40 = 9:1 (1000 mg/l) and laboratory water control.
Reference: EA, Japan (1992)

(b)
Type of test: static **[ ]**; semi-static **[ ]**; flow-through **[ ]**; other **[ ]**;
open-system **[ ]**; closed-system **[ ]**
Species: *Daphnia magna* Straus
Exposure period: 48 hrs
Results: EC_{50} (24h) >500 mg/l
EC_{50}(48h) >500 mg/l
EC_{0} (24h) = 500 mg/l
EC_{100}(24h) >500 mg/l
EC_{0} (48h) = 125 mg/l
EC_{100}(48h) >500 mg/l
NOEC =
LOEC =
Analytical monitoring: Yes [ ] No **[ ]** ? **[X]**
Method: EEC Directive 79/831, 1989
100 mg/l (Tween 80)
GLP: Yes **[ ]** No **[ ]** ? [X]
Remarks:
Reference: Company data (Germany)
BASF AG, Labor Oekologie; unpublished report (0712/88)

B. Other aquatic organisms

No studies located

4.3 TOXICITY TO AQUATIC PLANTS e.g. Algae

Species: *Selenastrum capricornutum* ATCC 22662

End-point: Biomass **[X]**; Growth rate **[ ]**; Other **[ ]**
Exposure period: 72 hours
Results: Biomass: $EC_{50\ (24h)}$ =
$EC_{50\ (72h)}$ = 6.5 mg/l
NOEC = 3.1 mg/l ($p < 0.05$)
LOEC =
Analytical monitoring: Yes **[ ]** No **[X]** ? **[ ]**
Method: OECD Test Guideline 201 (1984)
open-system **[**X**]**; closed-system **[ ]**
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Remarks: The EC_{50} values were calculated based on 5 nominal concentrations (1.8-18 mg/l), DMSO control and laboratory water control.
Reference: EA, Japan (1992)

(b)
Species: *Scenedesmus subspicatus*
End-point: Biomass **[ ]**; Growth rate **[ ]**; Other **[ ]**
Exposure period:
Results: EC_{50}(72h) = 5.3 mg/l
EC_{20}(72h) =<3.9 mg/l
EC_{90}(72h) = 27 mg/l
EC_{50}(96h) = 6.3 mg/l
EC_{20}(96h) = 3.9 mg/l
EC_{90}(96h) = 21 mg/l
NOEC =
LOEC =
Analytical monitoring: Yes **[ ]** No **[ ]** ? **[X]**
Method: DIN 38412 Part 9, 100 mg/l (Cremophor RH 40)
open-system [ ]; closed-system [ ]
GLP: Yes **[ ]** No [ ] ? **[X]**
Remarks:
Reference: Company data (Germany)
BASF AG, Labor Oekologie; unpublished report (0712/88)

4.4 TOXICITY TO BACTERIA

(a)
Type: Aquatic **[ ]**; Field **[ ]**; Soil **[ ]**; Other **[ ]**
Species: *Pseudomonas putida*
Exposure period:
Results: EC_{10}(30 min) =>10000 mg/l
Analytical monitoring: Yes **[ ]** No **[ ]** ? [X]
Method: DIN 38412 Part 27, 100 mg/kg (Tween 80)
GLP: Yes [ ] No **[ ]** ? **[X]**
Test substance: Indigo Blue
Remarks:
Reference: Company data (Germany)
BASF AG, Labor Oekologie; unpublished report (0712/88)

(b)
Type: Aquatic **[ ]**; Field **[ ]**; Soil **[ ]**; Other **[ ]**
Species: Activated sludge
Exposure period: 30 min.
Results: EC10(30 min) =>1000 mg/l
Analytical monitoring: Yes **[ ]** No **[ ]** ? **[X]**

Method:	ISO 8192, Test for inhibition of oxygen consumption by activated sludge
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Indigo Blue
Remarks:	
Reference:	Company data (Germany) BASF AG (1988), Labor Oekologie, unpublished report (347/88)

4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

4.5.1. CHRONIC TOXICITY TO FISH

No studies located

4.5.2. CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Type of test:	static **[ ]**; semi-static **[**X**]**; flow-through **[ ]**; other **[ ]**; open-system **[X]**; closed-system **[ ]**
Species:	*Daphnia magna*
End-point:	Mortality **[X]**; Reproduction rate **[X]**; Other **[ ]**
Exposure period:	21 day
Results:	
Mortality:	LC_{50} (24 h) = > 78 mg/l LC_{50} (48 h) = > 78 mg/l LC_{50} (96 h) = 140 mg/l (95% confidence level: 63-650 mg/l) LC_{50} (7 d) = 41 mg/l (95% confidence level: 26-80 mg/l) LC_{50} (14 d) = 5.5 mg/l (95% confidence level: 4.0-7.6 mg/l) LC_{50} (21 d) = 2.6 mg/l (95% confidence level: 1.9-3.4 mg/l) NOEC = LOEC =
Reproduction:	EC_{50} (14 d) = 1.9 mg/l EC_{50} (21 d) = 1.6 mg/l (95% confidence level: 1.3-2.0 mg/l) NOEC = 0.78 mg/l ($p < 0.05$) LOEC = 2.5 mg/l ($p < 0.05$)
Analytical monitoring:	Yes **[ ]** No **[X]** ? **[ ]**
Method:	OECD Test Guideline 202 (1984)
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Test substance:	Indigo Blue, purity = 96 %
Remarks:	40 daphnids (4 replicates; 10 organisms per replicate) were exposed to 5 nominal concentrations (0.78-78 mg/l), a vehicle control of DMSO:HCO-40 = 9:1 (1000 mg/l) and a culture medium control.
Reference:	EA, Japan (1992)

4.6 TOXICITY TO TERRESTRIAL ORGANISMS

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

No studies located

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

No studies located

4.6.3 TOXICITY TO OTHER NON MAMMALIAN TERRESTRIAL SPECIES (INCLUDING AVIAN)

No studies located

4.7 BIOLOGICAL EFFECTS MONITORING (INCLUDING BIOMAGNIFICATION)

No studies located

4.8 BIOTRANSFORMATION AND KINETICS IN ENVIRONMENTAL SPECIES

No studies located

4.9 ADDITIONAL REMARKS

None

5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

(a)

Type :	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rat
Value :	> 5,000 (mg/kg)
Method:	OECD Test Guideline
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Indigo Blue
Remarks:	
Reference:	Unpublished company data

(b)

Type :	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rat/SD
Value :	> 3,160 (mg/kg)
Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Indigo Blue
Remarks:	
Reference:	Ferber, K.H. (1987)

(c)

Type :	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Dog
Value :	> 1,000 (mg/kg)
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Indigo Blue
Remarks:	Unknown
Reference:	Ferber, K.H. (1987)

5.1.2 ACUTE INHALATION TOXICITY

(a)

Type :	LD_0 **[X]**; LD_{100} **[ ]**; LD_{50} **[ ]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rat (SD)
Value:	> 20% (0.08 mg/l)/4hrs
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	
Comments:	
Reference:	Ferber, K.H., J. Environ. Pathol. Toxicol. Oncol., 7(4), 73-84, 1987.

(b)

Type :	LD_0 **[X]**; LD_{100} **[ ]**; LD_{50} **[ ]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rat
Value:	5.3 mg/l/4hrs
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	
Comments:	
Reference:	Unpublished company data

5.1.3 ACUTE DERMAL TOXICITY

Type :	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rabbit/New Zealand White
Value:	> 20% (2000 mg/kg)
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[**X**]**
Test substance:	
Reference:	Ferber, K.H. (1987)

5.1.4 ACUTE TOXICITY, OTHER ROUTES OF ADMINISTRATION

No studies located

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

(a)

Species/strain:	Rabbit
Results:	Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[X]**
Classification:	Highly corrosive (causes severe burns) **[ ]**; Corrosive (caused burns) **[ ]**; Irritating **[ ]**; Not irritating **[ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	
Remarks:	
Reference:	Unpublished company data

(b)

Species/strain:	Rabbit
Results:	Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[X]**
Classification:	Highly corrosive (causes severe burns) **[ ]**; Corrosive (caused burns) **[ ]**; Irritating **[ ]**; Not irritating **[ ]**
Method:	OECD test Guideline
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Indigo liquid 20 %
Remarks:	
Reference:	Unpublished company data

5.2.2 EYE IRRITATION/CORROSION

Species/strain:	Rabbit
Results:	Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[X]**
Classification:	Highly corrosive (causes severe burns) **[ ]**; Corrosive (caused burns) **[ ]**; Irritating **[ ]**; Not irritating **[ ]**
Method:	BASF method
GLP:	Yes **[ ]** No **[ ]** ? **[ ]**
Test substance:	

Remarks:
Reference: Unpublished company data

5.3 SKIN SENSITISATION

Type: Patch-Test
Species/strain: Human
Results: Sensitizing **[ ]**; Not sensitizing **[X]**; ambiguous **[ ]**
Classification:
Sensitizing **[ ]**; Not sensitizing **[X]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ ]**
Test substance:
Remarks:
Reference: Unpublished company data

5.4 REPEATED DOSE TOXICITY

(a)
Species/strain: Rat (albino)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: oral (Diet)
Exposure period: 2 years
Frequency of treatment:
Post exposure observation period:
Dose: 0, 0.25, 1.0 or 3.0 % (0, 100, 400, 1200 mg/kg/day)
(25 animals/group)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
(80 males and 80 females)
NOEL:
LOEL:
Results: Appearance and behavior of the test rats were generally comparable to those of the controls. At the 3% level, food consumption was significant lower than controls for the first six months but comparable to controls during the remainder of the study. No significant difference in survival rats were noted. Significantly lower hematocrit and hemoglobin values in comparison with controls were obtained at all time intervals except 24 months for the males fed 3%. No such effect was noted in the females. Bilirubinuria was observed at 24 months in male and female at 1% and females at 3%. The test animals sacrificed at 52 weeks did not present any gross changes in the organs or viscera attributable to the test material. Autopsies performed on the animals which died during the second year of the study did not reveal any consistent gross changes. Microscopic findings at two years revealed no compound related effects on the kidneys or other tissues in either sex. The study appeared to demonstrate that after a period of adjustment to the higher dosage levels, the rats were able to tolerate up to 3% of this substance in their diets without serious effects.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Reference: Ferber, K.H. (1987)

(b)
Species/strain: Dog (beagle)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: oral (Diet)
Exposure period: 2 years
Frequency of treatment:
Post exposure observation period:
Dose: 0, 0.25, 1.0 or 3.0 % (3 animals/group)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL:
LOEL:
Results: No changes in appearance, behavior or elimination were deemed to be compound related and none were dose dependent. Most of the dogs maintained or gained body weight. Lost weights were not dose dependent. Clinical results were within normal limits. Scattered abnormal gross pathological findings did not appear to be compound related and organ weights were not significantly different from those of controls. No histological alterations were found beyond those disease processes usually found in laboratory dogs.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Reference: Ferber, K.H. (1987)

(c)
Species/strain: Rabbit
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Dermal
Exposure period: 13 weeks
Frequency of treatment: 5 days/week
Post exposure observation period:
Dose: 0, 500 mg/kg/day (1 - 4 animals/group)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL:
LOEL:
Results: Weight trends, mortality and signs of systemic toxicity and dermal irritation were recorded. Blood counts and urinalysis were performed on all animals at the start and termination of the study. Additional samples were taken during the fourth weeks from those animals tested for 13 weeks (64 applications). All animals were subjected at autopsy or death or terminal sacrifice. Tissues examined microscopically were: brain, thyroid, lung, heart, liver, kidneys, adrenal, skin and bone mallow.
No effects on any of the examined tissues, or outward signs (mortality, skin conditions) could be ascribes to the test compound.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Reference: Ferber, K.H. (1987)

5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

(a)
Type : Bacterial reverse mutation assay
System of testing:
Species/strain: *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538
Concentration:
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:
Cytotoxicity conc: With metabolic activation:
Without metabolic activation:
Precipitation conc:
Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[X]**
Without metabolic activation:	**[ ]**	**[ ]**	**[X]**

Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Teat substance:
Remarks:
Reference: Carcinogenesis (1982)
Muzzall, J.M. and Cook, W.L. (1979)
Jongen, W.M.F. and Alink, G.M. (1982)

(b)
Type : Bacterial reverse mutation assay
System of testing:
Species/strain: *S. typhimurium* TA 98
Concentration:
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:
Cytotoxicity conc: With metabolic activation:
Without metabolic activation:
Precipitation conc:
Genotoxic effects: Positive
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Teat substance: No high purified substance
Remarks:
Reference: Unpublished company data

B. NON-BACTERIAL IN VITRO TEST

Type : Cytogenetics Assay
System of testing: Species/strain: Chinese hamster CHL cells
Concentration: Incubated with 0, 124, 500, 1000 or 2500 µg/plate
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**;
No data **[ ]**
Results:
Cytotoxicity conc: With metabolic activation:
Without metabolic activation:
Precipitation conc: not stated
Genotoxic effects:

	+	?	-
With metabolic activation:	**[X]**	**[ ]**	**[ ]**
Without metabolic activation:	**[X]**	**[ ]**	**[ ]**

Method: Japanese Guideline for Screening Mutagenicity testing of chemicals
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance: Commercial, purity 97.2 %
Remarks: Plates/test:2

Activation system: S-9 fraction from the liver of Phenobarbital and 5,6-Benzoflavone induced male SD derived rats with NADPH-generating system
No. replicates: 1

Reference: MHW, Japan (1993) Unpublished Report on Mutagenicity Test of Indigo Blue. (HPV/SIDS Test conducted by MHW, Japan)

5.6 GENETIC TOXICITY IN VIVO

Type: Micronucleus Test
Species/strain: Mouse
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: oral; Gavage
Exposure period: two times (interval 24 hours)
Doses: 0, 0.1, 0.5, 1.0 and 2.0 mg/kg
Results:
Effect on mitotic index or P/N ratio:
Genotoxic effects: + ? -
[ ] [ ] [X]
Method: The animals were sacrificed at 30 and 54 hours after first administration. 1,000 Erythrocytes were examined from each animal. Cyclophosphamide was used as positive control.
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Unpublished company data

5.7 CARCINOGENICITY

Species/strain: Mouse
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Dermal
Exposure period: 95 weeks
Frequency of treatment: once/week
Postexposure observation period:
Doses: 1 % solution in benzene
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[X]**; Historical **[X]**
Results: No significant increases as compared to the no treatment group were observed.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: The negative result was not clear because of the effects of unusual vehicle.
Reference: Unpublished company data

5.8 TOXICITY TO REPRODUCTION

Type: Fertility **[ ]**; One generation study **[ ]**; Two generation study **[ ]**; Other **[X]** (Three generation study)
Species/strain: Rat (Harlan-Wistar)

Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: oral (Diet)
Exposure period:
Frequency of treatment:
Postexposure observation period:
Premating exposure period:
Duration of the test;
Doses: 0, 5, 50, 150 or 500 mg/kg
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**;
Historical **[ ]**
NOEL Parental :
NOEL F1 Offspring:
NOEL F2 Offspring:
Results: No deleterious effects were associated with the inclusion of D&C Blue No. 6 in the diet of rats for 3 generation.
Method: The procedure is described contractor (Carnege-Mellon Univ., 1973)
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: None
Reference: Ferber, K.H. (1987)

5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY

(a)
Species/strain: Rat/CD
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Oral (gavage)
Duration of the test;
Exposure period: From day 6 through day 15 of gestation
Frequency of treatment:
Doses: 0, 50, 160 or 500 mg/kg/day
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**;
Historical **[ ]** (Methyl cellulose)
NOEL Maternal Toxicity:
NOEL teratogenicity :
Results: On the basis of number of viable and dead fetuses, resorption site, mean fetal weight, sex distribution, mean litter size, frequency of anomalies or weight gain of pregnant females, D%C Blue No. 6 was without effect on reproduction performance, maternal weight gain and fetal development.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[**X**]**
Test substance:
Remarks:
Reference: Ferber, K.H. (1987)

(b)
Species/strain: Rabbit (New Zealand White)
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Oral
Duration of the test;

Exposure period: From day 6 through day 18 of gestation
Frequency of treatment: Every day
Doses: 0, 50, 160 or 500 mg/kg/day
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**;
Historical **[ ]**
NOEL Maternal Toxicity:
NOEL teratogenicity :
Results: Any abnormality was not observed in fetus treated with D & C Blue No. 6.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[**X**]**
Test substance:
Remarks:
Reference: Ferber, K.H. (1987)

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

No studies located

B. Toxicodynamics, toxicokinetics

No studies located

5.11 EXPERIENCE WITH HUMAN EXPOSURE

None

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