FOREWORD INTRODUCTION

IRON DICHLORIDE

CAS N°: 7758-94-3

SIDS Initial Assessment Report

For

SIAM 19

(Berlin, Germany, 19-22 October 2004)

1. Chemical Name:	Iron dichloride
2. CAS Number:	7758-94-3
3. Sponsor Country:	Republic of Korea Contact Point: Myungjin KIM, Ph. D. National Institute of Environmental Research. Kyungseo-dong, Seo-gu, Incheon, 404-708, Korea Tel: +82-(0)32-560-7216 FAX: +82-(0)32-560-7256
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 ?	This substance is sponsored by Korea. The assessment process was started in 1999. National Institute of Environmental Research of Korea conducted a literature search, reviewed submitted data and prepared documents for SIAM 19.
7. Review Process Prior to the SIAM:	National Institute of Environmental Research of Korea peer-reviewed the documents and evaluated the quality.
8. Quality check process:	National Institute of Environmental Research of Korea peer-reviewed selected endpoints and verified the data in SIDS dossier with original studies.
9. Date of Submission:	
10. Comments:	

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	7758-94-3
Chemical Name	Iron dichloride
Structural Formula	**Cl —— Fe —— Cl**

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

An acute oral toxicity study (Acute Toxic Class Method OECD TG 423) showed that the LD_{50} of iron dichloride was between 300 and 2000 mg/kg bw. All animals of the 2,000 mg/kg b.w. treatment group and one animal of the 300 mg/kg bw treatment group died. The necropsy showed hemorrage on lymphtic nodes, stomach, intestine and thymus, and hypertrophy of pancreas and spleen. In the 300 mg/kg bw group, the clinical signs such as hypoactivity and piloerection recovered within the test period. For humans, significant gastrointestinal manifestations occur following ingestion of 20 mg of elemental iron/kg bw while systemic toxicity may occur at 60 mg/kg bw. Generally, doses of various ferrous or ferric salts greater than 150 mg/kg bw of elemental iron are considered serious. According to an acute dermal toxicity study, the acute lethal dose (LD_{50}) was greater than 2,000 mg/kg bw. There was no animal death during the test.

The skin irritancy of iron dichloride was low. Only weak edema was induced on application sites. However, the effect of iron dichloride on the eyes was quite severe. In the early stages after application, opacity of cornea was observed and severe edema with redness and swelling were observed in conjunctiva. These pathological changes were not recovered within the test periods. Therefore, iron dichloride is a corrosive irritant to the eyes. No skin sensitization data are available.

In a repeated dose toxicity study performed according to OECD TG 422, Sprague-Dawley rats were treated orally at 0, 125, 250 and 500 mg/kg bw/day. The rate of body weight gain was decreased in males at 250 and 500 mg/kg bw/day compared to the control group. Black colored liver and hemorrhage with diffuse black pigmentation in the stomach were observed in male rats at 500 mg/kg bw/day. In males at 250 and 500 mg/kg bw/day, the organ weights of liver and adrenal glands were increased. According to these results, the NOAEL value was determined to be 125 mg/kg bw/day for male rats. In female at 500 mg/kg bw/day, three (out of 20) rats died during the treatment period. The liver weights and water consumption were increased and there were histopathological differences. Therefore the NOAEL value for female rats was 250 mg/kg bw/day.

In a genetic toxicity test (OECD TG 471), iron dichloride did not show mutagenic effects on *S. tryphimurium* (strains TA 98, TA 100, TA 1535 and TA 1537), and on *E. coli* WP2 *uvr*A up to 5,000 µg/plate. In an oral *in vivo* micronucleus assay (OECD TG 474), no increase of the micronucleus was observed when tested up to 50 mg/kg/day (MTD). Therefore, iron dichloride is not considered to be a mutagen.

A reproduction and developmental study on rats was also performed according to OECD TG 422. For the reproduction toxicity, there was no significant difference in mating data, pre and post implantation loss rate between the control group and the treatment groups. For the developmental study, mean litter size, birth rate, survival rate, and body weights of litters were not affected. In conclusion, the NOAEL of iron dichloride on reproduction and developmental toxicity was 500 mg/kg bw/day for both male and female rats.

Environment

Iron dichloride is a solid inorganic substance (white rhombohedral crystals), sometimes has a green tint and is very hygroscopic. Its commercial form is liquid. It is freely soluble in water with a solubility of 650 g/L at 25 °C. Vapor pressure, partition coefficient in n-octanol/water and stability in water according to OECD TG 111 are not applicable for the salt of an inorganic substance. Photodegradation and biodegradation are not relevant for an inorganic compound. Environmental fate modeling cannot be performed with the available data.

Bioaccumulation is not expected.

The following studies for aquatic organisms were performed:

Green algae (*Selenastrum capricornutum)*: EC_{r50} (72 h) = 6.9 mg/L (growth rate)
EC_{b50} (72 h) = 3.8 mg/L (biomass)
Invertebrates (*Daphnia magna):* EC_{50} (48 h) = 19.0 mg/L.
Fish (*Oryzias latipes*): LC_{50} (96 h) = 46.6 mg/L.

For fish and algae, the observed effects were partially due to the pH changes. For fish, no mortality was observed up to 100 mg/l of iron dichloride in neutralised solutions. For algae, test solutions dropped below pH 7 at concentrations of 12 mg/l and above.

No data were available on terrestrial organisms. From the results of aquatic organisms of three trophic levels, iron dichloride is considered to be moderately toxic in the aquatic environment.

Exposure

In Korea, the estimated production amounts of iron dichloride were approx. 100,000 tonnes/year in 1998. The European production capacity of iron dichloride is estimated to be 250,000 tonnes in 2004.

Iron dichloride is produced by reaction of scrap iron with waste liquid hydrochloric acid in a continuous closed reactor and this chemical is mainly used as a supplementary cohesion agent to treat dye wastewater in textile, dye and paper manufacturing industries and as a raw material for iron trichloride production in Korea. In Europe iron dichloride is used for water treatment, H_2S reduction, as a pigment and for soil immobilization. Further uses are as follows; metallurgy; reducing agent; pharmaceutical preparations; mordant in dyeing; sewage treatment.

Iron dichloride is produced in a closed system and wastewater containing this chemical is recycled in to the manufacturing process. In the wastewater plants of textile, dye and paper manufacturing industries, dye sewage is treated with iron dichloride. Ferrous ion is oxidized to ferric ion which precipitates to form a slurry. The slurry contains ferric hydroxide ($Fe(OH)_3$) and the supernatant of treated dye sewage is discharged. Therefore, environmental exposure of iron dichloride is expected to be very low and mostly ferric ion would be released.

As for human exposure, there is a potential for exposure to workers *via* inhalation and dermal routes during the packaging or processing the raw material, cleaning of reaction tank or filtration after the reaction. But occupational exposure is controlled with personal protective equipments like goggles and gas filter mask and with ventilation in Korea. The substance is not classified as a hazardous chemical which is monitored for workplace exposure in Korea annually. Therefore, monitoring data for occupational exposure is not available. ACGIH TLV of iron dichloride is TWA 1 mg (Fe)/m^3. In the manufacturing factories, workers may be exposed to the mist of hydrochloric acid but monitoring data by personal air sampling of hydrochloric acid were under TLV-ceiling of 5 ppm.

There is no direct use and there are no consumer products containing iron dichloride in Korea.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health: The chemical is currently of low priority for further work. The chemical possesses properties indicating a hazard for human health (corrosivity). The chemical is produced in a closed system and exposure to workers during the processing of the chemical is low. Based on data presented by Sponsor country (relating to labelling), adequate risk management measures are being applied. Countries may desire to check their own risk management measures to find out whether there is a need for additional measures.

Environment: The chemical is a candidate for further work. Based on the aquatic toxicity data and the use pattern presented by the sponsor country, member countries are invited to perform an exposure assessment, and if then necessary a risk assessment. Consideration should be given to the ongoing assessment of other iron salts in the OECD HPV Chemicals Programme.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number:	7758-94-3
IUPAC Name:	Iron dichloride
Molecular Formula:	FeCl2
Structural Formula:	**Cl —— Fe —— Cl**
Molecular Weight:	126.75
Synonyms:	Iron chloride ($FeCl_2$) Ferrous chloride Ferrous chloride ($FeCl_2$) Ferrous dichloride Iron protochloride Iron(2+) chloride Iron(II) chloride Iron(II) chloride ($FeCl_2$) Iron(II) chloride (1:2)

1.2 Purity/Impurities/Additives

Purity:	35.3 % (Liquid, The Commercial Product)
Impurity:	0.0005 % Lead (Pb) 0.00094 % Cadmium (Cd) 0.1 % Manganese (Mn) 0.1 % Free acid 0.1 % Iron trichloride (FeCl3) 16.9 % Iron (Fe) 0.00946 % Copper (Cu) 0.00443 % Nickel (Ni) > 40 % water
Additives:	

1.3 Physico-Chemical properties

The physico-chemical properties of the pure anhydride are described in table 1.

Table 1 Summary of physico-chemical properties for iron dichloride

Property	Value	Reference
Physical state	Solid Liquid (The Commercial Product)	
Melting point	No data available.	
Boiling point	No data available.	
Density	No data available.	
Vapour pressure	Not applicable for the salt of inorganic compounds.	
Water solubility	650 g/L at 25 °C Freely soluble in water	(1) (2)
Partition coefficient n-octanol / water (log value)	Not applicable for the salt of inorganic compounds.	

Iron dichloride is in the form of white rhombohedral crystals and sometimes it has a green tint and the substance is very hygroscopic. Iron dichloride dihydrate (FeCl2•2H2O) is in the form of white monoclinic crystals with a pale green tint and loses 1 H2O at 120 °C. Also the substance is reported to lose 1 H2O at 150 – 160 °C. Iron dichloride tetrahydrate (FeCl2•4H2O) is in the form of pale green to blue-green monoclinic crystals or cryst powder and loses 2 H2O at 105 – 115 °C (2).

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

In Korea, the estimated production volume of iron dichloride was approx. 100,000 tonnes/year and the estimated volume of the substance used was 113,956.5 tonnes/year in 1998 (3). In Nordic countries, the estimated production volume of iron dichloride was 178 tonnes/year in 2000 (4). the European production capacity of iron dichlroride is estimated to be 250,000 tonnes/year of which about 80,000 tonnes/year were produced by the steel industry in 2004 (5).

In Korea, iron dichloride is produced by reaction of scrap iron with waste liquid hydrochloric acid in a continuous closed reactor.

$Fe + 2HCl \rightarrow FeCl_2 + H_2$

The product is purified by filtration and transferred to a storage tank through a closed line. The final product of iron dichloride is liquid so it is transported by tank lorry (3).

The general uses of iron dichloride are as follows:

- metallurgy, reducing agent, pharmaceutical preparations, manufacture of iron trichloride; mordant in dyeing, sewage treatment (2), odor removal, textile impression pigment, ink and photoengraving (6), a hot bath in iron plating baths to yield more ductile deposits (7).

In Korea, iron dichloride is mainly used as a supplementary cohesion agent to treat dye wastewater in textile, dye and paper manufacturing industries. In the processing of wastewater treatment, iron dichloride is used as a chemical treatment for dye sewage (3).

In Europe iron dichloride is used for water treatment, H_2S reduction, pigment and soil immobilization (5).

Also, iron dichloride is used for iron trichloride production. Iron trichloride is produced by reaction of iron dichloride with chlorine gas in the continuous closed reactor.

$2FeCl_2 + 3Cl_2 \rightarrow 2FeCl_3 + 2Cl_2$ (3)

Iron trichloride is mainly used for treatment of sewage and industrial wastes, engraving, photography, and printed circuitry, and as condensation catalyst in friedel-crafts reactions, mordant, oxidizing, chlorinating. It is also used as a condensing agent, disinfectant, pigment, feed additive and for water purification (8).

2.2 Environmental Exposure and Fate

In the Sponsor country, environmental releases are unlikely to occur during industrial manufacturing of iron dichloride as these processes take place in a closed system. Also, wastewater containing iron dichloride is recycled in the manufacturing process. So emissions of wastewater and exhaust gases containing iron dichloride to the environment are not likely to occur in these industries.

Iron dichloride is produced by reaction of iron and hydrochloric acid. From the reactor, mist of hydrochloric acid may be released into the atmosphere. But hydrochloric acid is removed by a scrubber connected to the reactor and is not detected by air sampling. Hydrochloric acid is regulated as a hazardous chemical in Korea so environmental monitoring is performed annually in the chemical manufacturing facilities.

In the wastewater treatment plants of textile, dye and paper manufacturing industries, iron dichloride is stored in closed storage tanks and added through automated injection system.

In the three steps of sewage treatment, iron dichloride is added in the first step together with hydrogen peroxide.

$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH$

In the second step, ferric ions react with sodium hydroxide and then a polymer is injected in the final step. After the third waste water treatment step, the sewage is precipitated to form a slurry. The slurry contains ferric hydroxide ($Fe(OH)_3$) and the supernatant of treated dye sewage is discharged to downstream. So the environmental release of iron dichloride into water is expected to be very low and mostly ferric ion would be released (3).

In the EU, a solution of iron dichloride is obtained as a by-product from the chloride process for the production of titanium dioxide. Apart from wastes emitted to land, which are mainly, metal hydroxides to landfill, there are no direct emissions from the filtration, neutralisation and extration equipment used in the production of the ferrous chloride solution (5).

Iron dichloride is produced at Wacker-Chemie GmbH in a closed system and only as a solution in water. The solution is used as a precipitant in water treatment plants and the resulting sludge is removed by sedimentation. After a drying procedure it is stored in a special waste deposit (9).

Iron dichloride occurs in nature as the mineral lawrencite (2).

Photodegradation, and biodegradation are not relevant for an inorganic compound. Environmental fate modeling cannot be performed with the available data. Bioaccumulation is not expected.

A stability test in water according to the OECD TG 111 is not applicable for inorganic salts.

In aqueous solution, ferric and ferrous irons are hydrolysed to give various hydrolysis species and can form acidic solutions.

Hydrolysis reactions of iron ions:

Fe(II) hydrolysis

$Fe^{2+} + H_2O \leftrightarrow FeOH^+ + H^+$
$Fe^{2+} + 2\ H_2O \leftrightarrow Fe(OH)_2 + 2\ H^+$
$Fe^{2+} + 3\ H_2O \leftrightarrow (Fe(OH)_3)^- + 3\ H^+$
$Fe^{2+} + 4\ H_2O \leftrightarrow (Fe(OH)_4)^{2-} + 4\ H^+$
$3\ Fe^{2+} + 4\ H_2O \leftrightarrow (Fe_3(OH)_4)^{2+} + 4\ H^+$

Fe(III) hydrolysis

$Fe^{3+} + H_2O \leftrightarrow (FeOH)^{2+} + H^+$
$Fe^{3+} + 2\ H_2O \leftrightarrow (Fe(OH)_2)^+ + 2\ H^+$
$Fe^{3+} + 3\ H_2O \leftrightarrow Fe(OH)_3 + 3\ H^+$
$Fe^{3+} + 4\ H_2O \leftrightarrow (Fe(OH)_4)^- + 4\ H^+$
$Fe^{3+} + 2\ H_2O \leftrightarrow (Fe_2(OH)_2)^{4+} + 2\ H^+$ (10)

2.3 Human Exposure

2.3.1 Occupational Exposure

Iron dichloride is manufactured and processed in a closed system. Workers may be exposed to the substance *via* inhalation and dermal routes during the packaging or processing of the raw material, during cleaning of reaction tanks or filtration after the reaction. Occupational exposure is controlled with personal protective equipment like goggles and gas filter masks and with ventilation (scrubber). The substance is not classified as a hazardous chemical to be monitored annually for workplace exposure in Korea. Therefore, monitoring data for occupational exposure is not available. The ACGIH TLV of iron dichloride is TWA 1 mg (Fe)/m^3. In the manufacturing factories, workers may be exposed to the mist of hydrochloric acid but monitoring data by personal air sampling of hydrochloric acid were under the TLV-ceiling of 5 ppm (3).

2.3.2 Consumer Exposure

Iron dichloride is mainly used in wastewater treatment plants. There is no direct use and there are no consumer products containing iron dichloride. Consumer exposure to the substance is not expected in Korea.

Iron trichloride is used to treat iron-deficiency anaemia at doses of 350 to 700 mg daily in divided doses (equivalent to about 100 to 200 mg iron daily) (10).

3 HUMAN HEALTH HAZARD

3.1 Effects on Human Health

3.1.1 Toxicokinetics & Metabolism

No specific information is available with iron dichloride. Information regarding the handling of iron in general by the body is described below.

Intestinal mucosa is the principal site for absorption of iron (11). Generally, 2 – 15 % of iron is absorbed from the gastrointestinal tract by transferrin. Absorption occurs in two steps. Ferrous ion is absorbed from the intestinal lumen into the mucosal cells and then transferred to plasma (11, 12).

Usually, an adult human contains 3 – 5 g of iron. About 65 % of total body iron is found in blood as haemoglobin. About 10 % of body iron is found in myoglobin and iron-requiring enzymes. The rest of iron (25 %) constitutes the body iron pool as protein-iron complex (ferritin and hemosiderin), which are found in liver, bone marrow and spleen (11, 12).

Normally, excess ingested iron is excreted in bile, sweat, feces and urine. Total iron excretion is usually of the order of 0.5 mg/day (12).

3.1.2 Acute Toxicity

Acute Oral Toxicity

Studies in Animals

In the recent toxicity test performed by OECD TG 423 (Acute Toxic Class Method) with GLP control, the LD50 value for acute oral toxicity of rats was between 300 and 2,000 mg/kg body weight (13).

Three Sprague-Dawley female rats were tested at each step and a starting dose of 300 mg/kg b.w. was selected. There was no mortality in the first three rats dosed with 300 mg/kg b.w. To confirm the starting dosage, three more rats were tested. In the second 300 mg/kg b.w. group, one animal was dead after one day. At the limit dosage of 2,000 mg/kg b.w., all three rats died after one hour of administration.

There were no effects on body weight increase in all surviving animals. The typical clinical signs of tested animals at 2,000 mg/kg b.w. were nasal discharge, hypoactivity, piloerection, prone position, reddish change and edema on ears, fore-legs and hind-legs. At 300 mg/kg b.w., all animals showed hypoactivity and piloerection on day 1 and some animals showed soft stool on day 2. However, these clinical signs were recovered to the normal status from day 3.

The necropsy of the tested animals at 2,000 mg/kg b.w. showed hemorrhage on lymphatic nodes, stomach, intestine and thymus. Also, there was hypertrophy of pancreas and spleen. In one dead animal of the 300 mg/kg b.w. group, hemorrhage on lymphatic nodes and intestine was observed. There was no abnormality observed by microscopic examination of the surviving animals of the 300 mg/kg b.w. group after sacrifice on day 15.

Studies in humans

Acute iron poisoning by accidental ingestion of iron-containing medicine is one of the most common toxicologic emergencies in young children. Toxic doses of elemental iron range from 20 mg/kg b.w. to more than 60 mg/kg b.w. Iron exerts both local and systemic effect, which is a result of cell death and tissue damage caused by metabolic acidosis. Ingestion of more than 250 mg/kg b.w. of elemental iron is potentially lethal (12, 14).

Acute Dermal Toxicity

According to the dermal toxicity study performed by NIER (15), the acute lethal dose (LD_{50}) was greater than 2,000 mg/kg body weight. Following the test guideline (TG 402), the limit test (2,000 mg/kg b.w.) was performed in 5 male and 5 female Sprague-Dawley rats. Because there was no animal death during the test period, the study was concluded.

Yellowish-brown changes on the skin of applied sites were observed in all treated animals from day 2 but this was recovered during the test period, except for 3 females. 2 males and 4 females showed reddish nasal discharge on day 2. All rats gained normal body weight throughout the study. Regarding gross pathology, no abnormalities were observed in all animals. Only in one male and in one female, scars were observed on the application sites.

3.1.3 Repeated dose toxicity

A repeated dose toxicity study was conducted according to the OECD TG 422, Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Screening Test (16). Sprague-Dawley rats were orally administered with Iron dichloride at 0, 125, 250 and 500 mg/kg b.w./day. The exposure period was 42 days for male rats and 42 – 54 days for female rats.

Clinical signs such as blackish stool and salivation were observed during the test period, but these were recovered within the recovery period. While all male rats survived, there were three female deaths in the 500 mg/kg b.w./day group. Two of them were died on day 38 and on day 46 of the treatment period, and the third one was died during the recovery period, on day 51. The cause of death was gastrointestinal damages by the test substance. Water consumption was increased in animals of both sexes from the 500 mg/kg b.w./day groups.

The rate of body weight gain showed a statistically significant decrease in males of the 250 and 500 mg/kg b.w./day groups. For female rats, no dose-related change was observed. Cases of diffuse black colored liver and hemorrhage with diffuse black pigmentation in necropsy opinion were caused by the test substance in males of the 500 mg/kg b.w./day group, but these were recovered during the recovery period.

Absolute and relative weights of liver and adrenal glands were changed in males of the 250 and 500 mg/kg b.w./day groups and in females of the 500 mg/kg b.w./day group. Cases of hemosiderin deposit in hepatocyte and hyperplasia of zona fasciculate in adrenal cortex were observed. The sensory reactivity, the auricle reflex and corneal reflex tests, in male and female tested groups were not different from control groups. Also, there were no specific findings in urinalysis. In analysis of blood, statistically significant differences were found in mean cell volumes (MCV), eosinophils (EOS), platelets (PLT), cholinesterase (CS), and triglycerides (TG). But these were within the biologically normal range and there were no dose-dependent changes.

From these results, the NOAEL values were 125 mg/kg b.w./day for male rats and 250 mg/kg b.w./day for female rats.

3.1.4 Genetic toxicity or mutagenicity

3.1.4.1 *In vitro* Studies

3.1.4.1.1 Bacterial test

A bacterial reverse mutation test, according to OECD test guideline 471, was performed in compliance with GLP (17).

Preliminary experiments to find the dose range were carried out using the pre-incubation method with 5 % S9 mix as a metabolic activation system. Employed doses were 1.6, 8, 40, 200, 1,000 and 5,000 μg/plate, both in the absence and in the presence of a metabolic activation system. 5,000 μg/plate was chosen as the maximum test concentration.

In the main study, iron dichloride did not increase reverse mutations of *Salmonella tryphimurium* (strains TA 98, TA 100, TA 1535 and TA 1537) and *Escherichia coil* (strain WP2 *uvr*A) with and without a metabolic activation system at 33.3, 100, 300, 1,000, 3,000 and 5,000 μg/plate. There was no statistically significant difference up to the maximum test concentration of 5,000 μg/plate ($p > 0.01$). Precipitation was noted at doses greater than 300 μg/plate. It was concluded that iron dichloride did not exhibit mutagenic activity to any test strains under the test conditions.

Table 3-1 Result of bacterial reverse mutation assay with iron dichloride

Tester strain	Chemical treated	Dose (μg/plate)	Colonies/plate (mean ± SD)	
			Without S9 mix	With S9 mix
TA 98	Test item	0	30 ± 4	29 ± 8
		33.3	30 ± 6	24 ± 1
		100	29 ± 4	32 ± 10
		300	28 ± 4	26 ± 5
		1,000	18 ± 6 *	27 ± 7 *
		3,000	19 ± 4 *	26 ± 8 *
		5,000	6 ± 1 *	30 ± 11 *
TA 100	Test item	0	88 ± 13	97 ± 17
		33.3	67 ± 6	91 ± 8
		100	78 ± 3	75 ± 3
		300	72 ± 7	92 ± 14
		1,000	77 ± 8 *	82 ± 3 *
		3,000	61 ± 15 *	117 ± 12 *
		5,000	25 ± 2 *	111 ± 33 *
TA 1535	Test item	0	18 ± 3	12 ± 3
		33.3	10 ± 3	10 ± 2
		100	16 ± 4	15 ± 1
		300	12 ± 1	11 ± 2
		1,000	13 ± 0 *	10 ± 1 *
		3,000	9 ± 3 *	10 ± 4 *
		5,000	7 ± 2 *	12 ± 4 *
TA 1537	Test item	0	12 ± 3	11 ± 3
		33.3	21 ± 1	9 ± 4
		100	15 ± 8	7 ± 1
		300	9 ± 4	8 ± 4
		1,000	7 ± 4 *	10 ± 4 *
		3,000	3 ± 2 *	7 ±3 *
		5,000	3 ± 2 *	6 ± 3 *
E. coli WP2 *uvr*A	Test item	0	14 ± 1	14 ± 4
		33.3	12 ± 5	13 ± 2
		100	14 ± 3	14 ± 1
		300	11 ± 5	9 ± 2
		1,000	12 ± 0 *	13 ± 4 *
		3,000	12 ± 2 *	8 ± 1 *
		5,000	10 ± 4 *	12 ± 5 *

Positive control				
TA 98	2-NF	1.0	245 ± 24 SS	
	2-AA	2.0		631 ± 26 SS
TA 100	SA	0.5	404 ± 18 SS	
	2-AA	2.0		905 ± 31 SS
TA 1535	SA	0.5	338 ± 16 SS	
	2-AA	5.0		216 ± 16 SS
TA 1537	9-AA	50	276 ± 34 SS	
	2-AA	5.0		263 ± 24 SS
WP2 *uvr*A	4-NQ	2.0	598 ± 28 SS	
	2-AA	10		283 ± 28 SS

*; precipitation
SS; Statistical significance was observed ($p \leq 0.01$)
2-NF; 2-Nitrofluorene, 2-AA; 2-Aminoanthracene, SA; Sodium azide, 9-AA; 9-Aminoacridine, 4-NQ; 4-Nitroquinoline

3.1.4.2 *In vivo* Studies

A mammalian erythrocyte micronucleus test in accordance with OECD TG 474 was negative (18). Six mice per experimental and control groups were employed. Iron chloride was dissolved in corn oil, and administered to the mice by intraperitoneal injection. Corn oil and 0.2 mg/ml Mitomycin C in water were the negative and the positive control, respectively. All animals dosed with iron dichloride exhibited similar PCE / (PCE + NCE) ratios and MNPCE frequencies compared to the negative control group. It is concluded that iron dichloride did not induce gene mutation up to the test concentration of 50 mg/kg b.w./day.

Table 3-2 Summary of PCE / (PCE + NCE) ratio and MNPCE frequency

Treatment group	PCE / (PCE + NCE) (mean)	Group mean frequency of MNPCE per 2,000 PCE (mean ± S.D.)
Vehicle (10 ml/kg)	0.58	3.8 ± 2.3
Iron dichloride (12.5 mg/kg)	0.61	3.8 ± 1.6
Iron dichloride (25 mg/kg)	0.56	3.7 ± 1.5
Iron dichloride (50 mg/kg)	0.51	2.0 ± 1.0
Mitomycin C (2 mg/kg)	0.54	185.7 ± 16.3S

SS: Statistical significance was observed ($p \leq 0.05$)

Moreover, in a wing spot test with *Drosophila melanogaster*, trans-heterozygous larvae for the wing-hair mutations *mwh* and *flr* were orally treated at the third instar stage with iron dichloride. A negative result was obtained when the wings were inspected at the adult stage for spots expressing phenotypes of the markers (19).

3.1.5 Carcinogenicity

There is no information available on carcinogenicity.

3.1.6 Reproduction and Developmental Toxicity

A reproduction and developmental toxicity study of iron dichloride was performed in accordance with OECD TG 422 (16). Sprague-Dawley rats were treated orally at 0, 125, 250 and 500 mg/kg b.w./day. Male and female animals were dosed for 42 days and 42 – 54 days, respectively.

For the reproduction toxicity, there was no significant difference in mating data and in pre and post implantation loss rate between the control and the treatment groups. Therefore, NOAEL value was 500 mg/kg b.w./day for both sexes.

For the developmental study, the crown rump length (CRL) of female neonates on postpartum day 4 was significantly shorter in the 125 mg/kg b.w./day group. But the decrease did not correlate with bodyweight changes which is a main growth and developmental index. Furthermore, there was no dose-dependence of CRL decrease. Therefore, it was concluded that the test substance did not influence the growth of neonates. There was a case of acaudate in the 500 mg/kg b.w./day group. Because of the low frequency of occurrence, it was not a teratogenic effect. The NOAEL for the developmental toxicity was 500 mg/kg b.w./day.

3.1.7 Skin/Eye Irritation

External contact of the eye with acidic iron salts such as the sulfate or chloride has caused transient irritation and inflammation. On prolonged contact with the conjunctiva they have been known to cause a local brown discoloration. Skin and eye contact may produce severe irritation and burns (20).

An acute skin irritation study with iron dichloride was performed in rabbits according to the test guideline TG 404 (21). 0.5 g of iron dichloride was applied on the skin of male rabbits and the clinical signs were observed for 14 days.

There was no dead animal during the test period and a normal pattern of body weight increase was observed. However, there were erythema, scars and edema on the application sites of iron dichloride.

The eye irritation result was more severe. This toxicity test was performed according to test guideline TG 405 (22). Test animals were female rabbits. 0.1 g of iron dichloride was applied into a conjunctival sac of one eye and clinical signs were observed. Although there was no dead animal and a normal body weight increase was observed, there were quite severe clinical changes. Level 3 of opacity area (greater than three-quarters) was seen in cornea within 1 hour after the treatment. The degree of opacity increased to level 4 in 3 days and the iris was not discernible through the opacity. These cornea lesions did not recover within the test period.

The iris was also affected by iron dichloride. Hyperaemia and lack of light-reactivity were seen on day 2 after the treatment. Severe redness, edema and swelling were also observed in conjunctiva within 1 hour. This swelling caused partial eversion of lids and more than half of the lids were closed. Although the pathological lesions were recovered partially, the eyelids were transformed abnormally.

The results of the histopathological examination showed lymphocyte infiltration in the conjunctiva and severe granulomatous legions in the cornea. A purulent inflammation and corneitis were also observed in stroma of the cornea. These symptoms were not reversible within the test period. However, the clinical signs observed in the early state were recovered within 21 days because the histopathological changes were not observed in the iris.

As the result, iron dichloride caused severe corrosive effects on eyes.

3.1.8 Skin Sensitization

No skin sensitisation data are available.

3.1.9 Experience of Human exposure

The chelating properties of ethylenediaminetetra acetic acid (EDTA) led to its use in acute iron poisoning, but reported results varied widely. Since the introduction of the more specific iron chelating agents DTPA and desferrioxamine, reports of their use in the treatment of this condition have been favourable. One case of death from acute iron poisoning despite early treatment with EDTA was reported, and two cases in which a successful outcome followed the use of desferrioxamine and DTPA respectively (28).

When ferrous compounds are given orally, damage to the gastrointestinal tract has been demonstrated with small hemorrhagic spots and some edema in stomach and more severe changes in the intestine, especially the duodenum, with large hemorrhages, shrinkage of the villi and severe necrosis of the mucosa at autopsy in patients, children and adults, dying after ingestion of such iron compounds.

Two cases of acute iron intoxication, one without complications and the other with hepatic involvement and hemolysis, which responded successfully to treatment with desferrioxamine were described (29).

3.2 Initial Assessment for Human Health

The LD50 by acute oral toxicity test of iron dichloride for rats was between 300 and 2,000 mg/kg b.w. that showed moderate toxicity. However, iron dichloride was less toxic by dermal application with the LD50 greater that 2,000 mg/kg. The erythma and edema induced on the application sites were very weak and recovered easily. Therefore, the skin irritancy was low.

Iron dichloride showed corrosive effects on the eyes. The pathological effects appeared immediately after the exposure. Severe opacity of cornea was induced and was not recovered within the test period. Although Grade 4 edema in conjunctiva was recovered within the test period, morphological changes occurred. Therefore, iron dichloride is a corrosive irritant to eyes.

The NOAEL values for the repeated toxicity were 125 mg/kg b.w./day in male rats and 250 mg/kg b.w./day in female rats, the changes of body weights, clinical signs, water consumption, organ weights, necropsy opinions, and histopathology were the determining factors for the NOAEL.

Regarding reproduction and developmental toxicity, there was no significant difference up to the maximum test concentration between the control and the test groups. The NOAEL was 500 mg/kg b.w./day for both sexes.

Iron dichloride was not a mutagen based on the results from a bacterial reverse mutation test and a mammalian erythrocyte micronucleus test.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Iron dichloride is freely soluble in aqueous media. When ferrous ions react with hydroxide ions in water, solid ferrous hydroxide compounds such as $Fe(OH)_2(s)$ are formed. $Fe(OH)_2(s)$ reacts with oxygen and it changes to $4Fe(OH)_3(s)$. This reaction can be written as follows.

$FeCl_2 + 2H_2O \rightarrow Fe(OH)_2 + 2HCl$

$Fe^{2+}(aq) + 2OH^-(aq) \rightarrow Fe(OH)_2(s)$ (green)

$4Fe(OH)_2(s)$ (green) $+ O_2(g) + 2H_2O \rightarrow 4Fe(OH)_3(s)$ (red-brown) (23)

aq = aquatic, s = solid, g = gas

These reactions usually occur slowly in pure water but several inorganic compounds in the test media act as catalysts to process this reaction rapidly. 10 mg/L of iron dichloride in distilled water and in three types of test media (culture media for fish, daphnia and algae) were tested for precipitation. Precipitation occurred in all test media except distilled water, but the solution using distilled water turned to pale yellow after several days. Iron dichloride exists in several forms as ferric ion, chloride ion and ferrous hydroxides after dissolution in aqueous solution. Iron concentrations in the test solutions were analysed with ICP-AES. The results were obtained from the analyses of concentrations of iron dichloride in the test solutions from the fish toxicity test at 0, 48, 96 hours from the initiation of the study. Because of precipitation, the test solutions were mixed well before sampling. Total iron concentrations in the test solutions were converted into iron dichloride concentrations. The measured concentrations of fish toxicity test were in the range of 70 – 140 % of nominal concentrations.

The measured concentrations in the test solutions of the daphnia toxicity study during 48 hours were 97 – 112 % of the nominal concentrations and test solutions of the algae toxicity study during 72 hours were 75 – 85 %. So, measured concentrations were used in these tests instead of nominal concentrations.

The following acute toxicity results with aquatic organisms were obtained;

Table 4-1 Effects of Iron dichloride on aquatic organisms

Organisms	Species	Results	Test condition	Reference
Fish	*Oryzias latipes*	LC_{50} (96 h) = 46.6 mg/L	OECD TG 203 (static, measured concentration)	(24)
Invertebrate	*Daphnia magna*	EC_{50} (48 h) = 19.0 mg/L	OECD TG 202 (static, measured concentration)	(25)
Algae	*Selenastrum capricornutum*	EC_{r50} (72 h) = 6.9 mg/L EC_{b50} (72 h) = 3.8 mg/L $NOEC_r$ = 2.4 mg/L $NOEC_b$ = 1.1 mg/L	OECD TG 201 (static, measured concentration)	(26)

All of the aquatic acute tests were performed in accordance with the principles of GLP. In acute toxicity tests of fish and algae, the test solution became acidic. A preliminary test was conducted to show the effect of pH on fish. 100 mg/L of iron dichloride was dissolved and the pH of the test solutions was adjusted to 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0. The mortality was affected at pH 3.5 only. The Increase of mortality at 96 hours was assumed to be due to the lower pH gradient. In case of algae, the test solutions of 12, 22 and 44 mg/L concentration became acidic after 72 hrs. The acidic condition of test solution affected the growth of algae. Different from fish and algae, the pH of the test solutions for daphnia remained neutral due to the bufferring action of the M4 medium (24, 25, 26).

The effect of iron dichloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) on reproduction of *Salmo gairdneri* for was studied *in vitro*. Spermatozoa, ova and fertilization were tested according to the method by R. Billard and P. Roubaud (1985). The gametes were exposed independently for 40 min to iron

dichloride tetrahydrate added into diluents. The ovum diluent (insemination diluent, ID) was a NaCl solution (osmotic pressure: 250 mOsm kg^{-1}, pH 9.0, Tris 20 mM, glycine 50 mM). The spermatozoa were diluted at 10^{-1} and 10^{-2} of ID and the same diluent with K^+ added; they were immobile in this diluent (a conservation diluent). Insemination was carried out after the gametes had been washed. Ova and spermatozoa were mixed together in ID containing the test chemical (insemination test). Iron dichloride tetrahydrate in solution had toxic effects on spermatozoa from less than the concentration of 0.005 mg/L at an insemination dilution of 10^{-2} and from 0.73 mg/L at an insemination dilution of 10^{-1}. A similar sensitivity was found for ova (from 0.73 mg/L) and a higher sensitivity for fertilization (from 0.08 mg/L) (27).

4.2 Terrestrial Effects

No data is available on terrestrial organisms.

4.3 Other Environmental Effects

Several aquatic toxicity studies for iron salts are as follows;

Ferric chloride; *Brachydanio rerio*: LC_0 (48 hr) = 92.8 mg/L
Poecillia reticulata: LC_{50} (48 hr) = 117 mg/L
Oryzias latipes: LC_{50} (48 hr) = 23 mg/L
Daphnia magna: LC_{50} (48 hr) = 21 – 30 mg/L
Chlorella vulgaris: NOEC (120 day) = 2.7 mg/L
LOEC (120 day) = 5.45 mg/L

Ferric sulphate; *Oncorhynchus mykiss:* LC_{50} (96 hr) = 45.8 mg/L
(> 100 mg/L with pH adjustment)
Daphnia magna: LC_{50} (96 hr) = 11.48 mg/L Fe
Scenedesmus sp.: NOEC (7 day) = 5.6 mg/L

Ferrous sulphate; *Oncorhynchus mykiss*:LC_{50} (96 hr) = 45 mg/L
(pH 6.9 based on nominal addition)
Oncorhynchus mykiss:LC_{50} (96 hr) = 10.8 mg/L
(pH 6.9 based on analysised Fe^{2+} in solution at end point)
Daphnia magna: LC_{50} (48 hr) = 19.4 mg/L
Daphnia magna: LC_{50} (48 hr) = 35.1 mg/L
Chlorella vulgaris: NOEL (30 day) = 700 mg/L (10)

4.4 Initial Assessment for the Environment

Iron dichloride is freely soluble in water and due to its inorganic properties no data were applicable for vapour pressure and partition coefficient in n-octanol/water. Photodegradation, and biodegradation are not relevant for an inorganic compound. Environmental fate modelling cannot be performed with the available data. Bioaccumulation is not expected.

The following acute toxicity test results with aquatic organisms are available:

Oryzias latipes: LC_{50} (96 h) = 46.6 mg/L

Daphnia magna: EC_{50} (48 h) = 19.0 mg/L

Selenastrum capricornutum: EC_{r50} (72 h) = 6.9 mg/L, EC_{b50} (72 h) = 3.8 mg/L

For fish and algae, the observed effects are partially due to pH effects. For fish, no mortality was observed up to 100 mg/l in a neutralised solution. For algae, test solutions dropped below pH 7 at test concentrations of 12 mg/l and above.

No data are available for terrestrial organisms. From results of aquatic organisms of three trophic levels, iron dichloride is considered to be moderately toxic in aquatic environment.

5 RECOMMENDATIONS

Human Health: The chemical is currently of low priority for further work. The chemical possesses properties indicating a hazard for human health (corrosivity). The chemical is produced in a closed system and exposure to workers during the processing of the chemical is low. Based on data presented by Sponsor country (relating to labelling), adequate risk management measures are being applied. Countries may desire to check their own risk management measures to find out whether there is a need for additional measures.

Environment: The chemical is a candidate for further work. Based on the aquatic data and the use pattern presented by the sponsor country, member countries are invited to perform an exposure assessment, and if then necessary a risk assessment. Consideration should be given to the ongoing assessment of other iron salts in the OECD HPV Chemicals Programme.

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SIDS DOSSIER

Iron dichloride

CAS No. : 7758-94-3

Sponsor Country: Republic of Korea

Date of submission to OECD: July 2004

1. GENERAL INFORMATION

1.01 SUBSTANCE INFORMATION

*A.	**CAS number**	:	7758-94-3
B.	**Name (Primary name)**	:	
*C.	**Name (OECD name)**	:	Iron dichloride
†D.	**CAS Descriptor**	:	
E.	**EINECS-Number**	:	231-843-4
F.	**Molecular Formula**	:	$FeCl_2$
*G.	**Structural Formula**	:	

Cl —— Fe —— Cl

H. Substance Group :

I. Substance Remark (Indicate the substance remark as prescribed in the EINECS Inventory, if possible)

J. Molecular Weight : 126.75

1.02 OECD INFORMATION

A. Sponsor Country : Republic of Korea

B. Lead Organisation : National Institute of Environmental Research

Contact person	:	Myungjin Kim, Ph. D.
Address		Environmental Research Complex
Street	:	Kyungseo-dong, Seo-gu
Postal code	:	404-708
Town	:	Incheon
Country	:	Republic of Korea
Tel	:	+82-(0)32-560-7216
Fax	:	+82-(0)32-560-7256
E-mail	:	kimmj4@me.go.kr

C. Name of responder (Information on a responder should be provided when companies respond to Lead Organisation or SIDS Contact Points.)

Name	:	Same as above
Address	:	Same as above

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance : Inorganic

B. Physical State (at 20 °C and 1.013 hPa)
: Solid
Liquid (The Commercial Product)

C. Purity : 35.3 %

Remark :

Molecular formula	Chemical name	CAS No.
$FeCl_2 \cdot 2\ H_2O$	Iron dichloride dihydrate	16399-77-2
$FeCl_2 \cdot 4\ H_2O$	Iron dichloride tetrahydrate	13478-10-9
$FeCl_2 \cdot 6\ H_2O$	Iron dichloride hexahydrate	18990-23-3
$FeCl_3$	Iron trichloride	7705-08-0
$FeCl_3 \cdot 6\ H_2O$	Iron trichloride hexahydrate	10025-77-1

Source : Sam-Woo Chemical Corporation, Korea

1.2 SYNONYMS

: Iron chloride ($FeCl_2$)
Ferrous chloride
Ferrous chloride ($FeCl_2$)
Ferrous dichloride
Iron protochloride
Iron(2+) chloride
Iron(II) chloride
Iron(II) chloride ($FeCl_2$)
Iron(II) chloride (1:2)

1.3 IMPURITIES

CAS No : 7439-92-1
EINECS No : 231-100-4
Name : Lead (Pb)
Value : 0.0005 %
Source : Sam-Woo Chemical Corporation, Korea

CAS No : 7440-43-9
EINECS No : 231-152-8
Name : Cadmium (Cd)
Value : 0.00094 %
Source : Sam-Woo Chemical Corporation, Korea

CAS No : 7439-96-5
EINECS No : 231-105-1
Name : Manganese (Mn)
Value : 0.1 %
Source : Sam-Woo Chemical Corporation, Korea

CAS No :
EINECS No :
Name : Free acid
Value : 0.1 %
Source : Sam-Woo Chemical Corporation, Korea

CAS No : 7705-08-0
EINECS No : 231-729-4
Name : Iron trichloride ($FeCl_3$)

Value	:	0.1 %
Source	:	Sam-Woo Chemical Corporation, Korea
CAS No	:	7439-89-6
EINECS No	:	231-096-4
Name	:	Iron (Fe)
Value	:	16.9 %
Source	:	Sam-Woo Chemical Corporation, Korea
CAS No	:	7440-50-8
EINECS No	:	231-159-6
Name	:	Copper (Cu)
Value	:	0.00946 %
Source	:	Sam-Woo Chemical Corporation, Korea
CAS No	:	7440-02-0
EINECS No	:	231-111-4
Name	:	Nickel (Ni)
Value	:	0.00443 %
Source	:	Sam-Woo Chemical Corporation, Korea
CAS No	:	
EINECS No	:	
Name	:	Water
Value	:	> 40 %
Source	:	Sam-Woo Chemical Corporation, Korea

1.4 ADDITIVES

1.5 QUANTITY

Estimated production	:	Estimated production volume of iron dichloride was approx.100,000 tonnes/year and estimated usage of the substance was 113,956.5 tonnes/year in Korea in 1998. In Nordic countries estimated production volume of iron dichloride was 178 tonnes/year in 2000. European capacity of iron dichloride is estimated to be 250,000 tonnes of which about 80,000 tonnes is produced by the steel industry in 2004 report.
Remarks	:	(1), (2), (3)

1.6.1 LABELLING

1.6.2 CLASSIFICATION

Classified	:	Listed on the TSCA Inventory
Reason for regulation	:	
Remarks	:	(4)
Classified	:	Listed on Canadian Domestic Substance List (DSL)
Reason for regulation	:	
Remarks	:	

(4)

Classified : Listed on Australian Inventory of Chemical Substances (AICS)
Reason for regulation :
Remarks :

(4)

Classified : 8: corrosive material
Reason for regulation : 49 CFR Package Group: II
Remarks :

(5)

1.6.3 SHIPPING REGULATIONS

Transport Area	Proper Shipping Name	Hazard Class		Packaging Group	Quantity limitation
Domestic (Land, D.O.T.)	Iron dichloride, solid	8	NA1759	II	12 kg
	Iron dichloride solution	8	NA1760	II	1 L
International (Water, I.M.O.)	Corrosive solid, acidic, inorganic, N.O.S. (Iron dichloride)	8	UN3260	II	12 kg
International Air (I.C.A.O)	Corrosive solid, n.o.s.		UN1759		
	Corrosive liquid, n.o.s.		UN1760		

(6), (7)

1.7 USE PATTERN

Type : Type
Category : Non dispersive use

Type : Type
Category : Wide dispersive use

Type : Industrial
Category : In metallurgy

(8)

Type : Industrial
Category : Reducing agent

(8)

Type : Industrial
Category : In pharmaceutical preparations

(8)

Type : Industrial
Category : Manufacture of ferric chloride

(8)

Type : Industrial
Category : Mordant in dyeing

(8)

Type : Industrial
Category : Sewage treatment

(8)

Type : Industrial
Category : Supplementary cohesion agent in sewage treatment
(1)

Type : Industrial
Category : As odor removal
(9)

Type : Industrial
Category : As textile impression pigment
(9)

Type : Industrial
Category : In ink and photoengraving
(9)

Type : Industrial
Category : As a hot bath in iron plating baths to yield a more ductile deposit
(10)

Type : Industrial
Category : Additives for municipal sewerage systems to reduce the H_2S odour
(3)

Type : Industrial
Category : As a raw material for use in the production of iron oxide pigments.
(3)

Type : Industrial
Category : Immobilisation of the elements As, Cr, V and Cd in soil.
(3)

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUE

Exposure limit value
Type : ACGIH TLV
Value : TWA 1 mg(Fe)/m^3
(11)

Exposure limit value
Type : OSHA PEL
Value : TWA 1 mg(Fe)/m^3
(11)

Exposure limit value
Type : MSHA STANDARD - air
Value : TWA 1 mg(Fe)/m^3
(10)

Exposure limit value
Type : OEL - DENMARK
Value : TWA 1 mg(Fe)/m^3 JAN 1993
(12)

Exposure limit value
Type : OEL - NORWAY
Value : TWA 1 mg(Fe)/m^3, JAN1999
(12)

Exposure limit value
Type : OEL - FINLAND
Value : TWA 1 mg(Fe)/m^3 JAN 1993
(12)

Exposure limit value
Type : OEL-THE NETHERLANDS
Value : TWA 1 mg(Fe)/m^3 JAN 1993
(12)

Exposure limit value
Type : OEL - SWITZERLAND
Value : MAK- week 1 mg(Fe)/m^3, JAN1999
(12)

Exposure limit value
Type : OEL - UNITED KINGDOM
Value : LTEL 1 mg(Fe)/m^3, short term exposure limit 2 mg(Fe)/m^3, JAN1993
(12)

Exposure limit value
Type : OEL - IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN, KOREA
Value : ACGIH TLV: TWA 1 mg(Fe)/m^3
(12)

Exposure limit value
Type : OEL - IN NEW ZEALAND, SINGAPORE, VIETNAM
Value : ACGIH TLV: TWA 1 mg(Fe)/m^3
(12)

1.9 SOURCES OF EXPOSURE

Source : Production and processing
Remarks : In Korea Iron dichloride is produced by reaction of scrap iron with waste liquid hydrochloric acid in the continuous closed reactor.

$Fe + 2HCl \rightarrow FeCl_2 + H_2$

The product is purified by filtration and transferred to a storage tank through a closed line. The final product of iron dichloride is a liquid so it is transported by tank lorry.
In the Sponsor country, environmental releases are unlikely to occur during industrial manufacturing of iron dichloride as these processes take place in a closed system. Also, wastewater containing iron dichloride is recycled in manufacturing process. So emissions of wastewater and exhaust gases containing iron dichloride to environment are not likely to occur in these industries.
In the manufacture, iron dichloride is produced by reaction of iron and hydrochloric acid. From the reactor, mist of hydrochloric acid may be released into the atmosphere. But hydrochloric acid is removed by scrubber connected to the reactor and is not detected by air sampling. Hydrochloric acid is a hazardous chemical in Korea so environmental monitoring is performed annually in the chemical manufacturing facilities.
(1)

Source : User

Remarks : In the wastewater treatment plants of textile, dye and paper manufacturing industries, iron dichloride is stored in the closed storage tank and added through automated injection system. In the three steps of sewage treatment, iron dichloride is added in the first step together with hydrogen peroxide.

$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH$

In the second step, ferric ion is reacted with sodium hydroxide and then polymer is injected in the final step. After the third waste water treatment step, the sewage is precipitated to form slurry. The slurry contains ferric hydroxide($Fe(OH)_3$) and the supernatant of treated dye sewage is discharged to downstream. So environmental exposure of iron dichloride into water is expected to be very low and mostly ferric ion would be released.

(1)

Source : Production and processing
Wacker-Chemie GmbH Burghausen
Remarks : Iron dichloride is produced at Wacker-Chemie GmbH in a closed system and only as a solution in water. Exposure to workers is therefore not anticipated. The solution is used as a precipitant in water treatment plants and the resulting sludge is separated by sedimentation. After drying it is stored in a special waste deposit.

(13)

Source : Occurs in nature as the mineral lawrencite.
Remarks :

(8)

Source : Production and processing
Kronos International, Inc
Remarks : In the EU, a solution of iron(II) chloride is obtained as a by-product from the chloride process for the production of titanium dioxide. Apart from wastes emitted to land, which are mainly metal hydroxides to landfill, there are no direct emissions from the filtration, neutralisation and extration equipments used for the production of the ferrous chloride solution.

(3)

Source : Consumer Exposure
Remarks : Iron(III) chloride is used to treat iron-deficiency anaemia at doses of 350 to 700 mg daily in divided doses (equivalent to about 100 to 200 mg iron daily).

(14)

1.10 ADDITIONAL REMARKS

Remarks : Water pollution
Harmful to aquatic life in very low concentrations.
May be dangerous if it enters water intakes.

(5)

2. PHYSICO-CHEMICAL DATA

2.1 MELTING POINT

Value : 677 °C
Reliability : (2) Reliable with restrictions
(15)

Value : 674 °C
Reliability : (2) Reliable with restrictions
(8)

Value : 670 - 674 °C
Reliability : (4) Not assignable
(16)

Value : 676 °C
Reliability : (4) Not assignable
(11)

2.2 BOILING POINT

Value : 1,023 °C
Decomposition :
Reliability : (2) Reliable with restrictions
(8), (15)

Value : 1,012 °C
Decomposition :
Reliability : (4) Not assignable
(11)

2.3 DENSITY (RELATIVE DENSITY)

Type : Density
Value : 3.16 g/cm^3
Temperature : 25 °C
Reliability : (2) reliable with restrictions
(8), (16)

Type : Density
Value : 3.16 g/cm^3
Temperature : No data
Reliability : (4) Not assignable
(11), (15)

2.4 VAPOUR PRESSURE

Value : 10 mmHg
Temperature : 700 °C
Reliability : (4) Not assignable

(11)

2.5 PARTITION COEFFICIENT $LOG_{10} P_{OW}$

Log P_{ow}	:	Not applicable for the salt of inorganic compounds.
Temperature	:	
Method	:	
Remarks	:	
Reliability	:	

2.6 WATER SOLUBILITY

Value	:	650 g/L
Temperature	:	25 °C
Reliability	:	(2) Reliable with restrictions
Flag	:	Critical study for SIDS endpoint

(15)

Value	:	No data
Temperature	:	No data
Remark	:	Freely soluble in water
Reliability	:	(2) Reliable with restrictions
Flag	:	Critical study for SIDS endpoint

(8)

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

Results	:	Not pertinent
Reliability	:	(4) Not assignable

(5)

2.9 FLAMMABILITY

Results	:	Not flammable
Reliability	:	(4) Not assignable

(5)

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

Value	:	Readily oxidized
Reliability	:	(4) Not assignable

(16)

2.12 OXIDATION: REDUCTION POTENTIAL

Value	:	0.771 V: $Fe^{3+} + e^{-} = Fe^{2+}$
Temperature	:	25 °C
Reliability	:	(2) Reliable with restrictions

(17)

2.13 ADDITIONAL DATA

3. ENVIRONMENTAL FATE AND PATHWAYS

3.1 STABILITY

3.1.1 PHOTODEGRADATION

Remarks : Not applicable for the salt of inorganic compounds
Reliability :

3.1.2 STABILITY IN WATER

Remarks : In water, exposed to air, or in solutions containing dissolved oxygen, iron dichloride oxidizes to iron trichloride and ferric oxide. Iron dichloride and iron trichloride are freely soluble. In solutions containing dissolved oxygen, a secondary reaction oxidizes the ferrous hydroxide to a ferric state. In the presence of hydroxyl ions in a neutral or slightly alkaline solution, this hydrated ferric hydroxide is precipitated.
Reliability : (4) Not assignable

(18), (19)

Remarks : Ferrous hydroxide is converted to ferric hydroxide in aqueous condition.
$Fe^{2+}(aq) + 2OH^{-}(aq) \rightarrow Fe(OH)_2(s)$ (green)
$4Fe(OH)_2(s)$ (green) $+ O_2(g) + 2H_2O \rightarrow 4Fe(OH)_3(s)$ (red-brown)
Reliability : (4) Not assignable

(20)

Remarks : <u>Hydrolysis reactions of iron ions:</u>

Fe(II) hydrolysis
$Fe^{2+} + H_2O \leftrightarrow FeOH^{+} + H^{+}$
$Fe^{2+} + 2\ H_2O \leftrightarrow Fe(OH)_2 + 2\ H^{+}$
$Fe^{2+} + 3\ H_2O \leftrightarrow (Fe(OH)_3)^{-} + 3\ H^{+}$
$Fe^{2+} + 4\ H_2O \leftrightarrow (Fe(OH)_4)^{2-} + 4\ H^{+}$
$3\ Fe^{2+} + 4\ H_2O \leftrightarrow (Fe_3(OH)_4)^{2+} + 4\ H^{+}$

Fe(III) hydrolysis
$Fe^{3+} + H_2O \leftrightarrow (FeOH)^{2+} + H^{+}$
$Fe^{3+} + 2\ H_2O \leftrightarrow (Fe(OH)_2)^{+} + 2\ H^{+}$
$Fe^{3+} + 3\ H_2O \leftrightarrow Fe(OH)_3 + 3\ H^{+}$
$Fe^{3+} + 4\ H_2O \leftrightarrow (Fe(OH)_4)^{-} + 4\ H^{+}$
$Fe^{3+} + 2\ H_2O \leftrightarrow (Fe_2(OH)_2)^{4+} + 2\ H^{+}$

Reliability : (4) Not assignable
Flag : Critical study for SIDS endpoint

(14)

Remarks : For iron artifacts buried in the ground, pitting is a prominent feature of the corrosion process. This anaerobic environment tends to be chemically reducing and forms soluble ferrous ions, which often diffuse some distance away from the iron surface. When iron is buried in an aerobic soil or exposed on the surface to the air, the ferrous ions initially formed in the corrosion process oxidize to ferric ions, resulting in layers of ferric oxide scale on the metal surface. This ferric oxide scale tends to form layers that may crack and spall due to the differences in the thermal expansion coefficients between the ferrous and ferric corrosion products and the metal. Alternatively, the corrosion products may inhibit additional corrosion by forming a protective film. Air-oxidized artifacts occupy more volume than the original metal and usually have obvious layers of ferric oxide scale. If salts, such as sodium chloride, are present in the environment, a very conductive solution is formed, and electrochemical corrosion is accelerated.
Reliability : (4) Not assignable

(19)

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA (ENVIRONMENT)

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

3.3.1 TRANSPORT

Remarks : Most of the iron comes into the seawater through the atmosphere, which, in turn receives it from dust. In atmospheric water droplets, a significant fraction of Fe(III) becomes solubilized and reduced to soluble Fe(II). In seawater the thermodynamically stable oxidation state is Fe(III).
Reliability : (4) Not assignable

(21)

3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Remarks : Environmental fate modeling cannot be performed with the available data.
Reliability :

3.4 IDENTIFICATION OF MAIN MODE OF DEGRADABILITY IN ACTUAL USE

3.5 BIODEGRADATION

Remarks : Not applicable for the salt of inorganic compounds
Reliability :

3.6 BOD5, COD OR RATIO BOD5/COD

3.7 BIOACCUMULATION

Remarks	:	Not applicable for the salt of inorganic compounds
Reliability	:	

3.8 ADDITIONAL REMARKS

4. ECOTOXICOLOGICAL DATA

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	Static
Species	:	*Oryzias latipes*
Exposure period	:	96 hours
Unit	:	mg/L
Analytical monitoring	:	Yes
LC_{50}	:	46.6 mg/L
Method	:	OECD TG 203 “Fish, Acute Toxicity Test”
Year	:	2004
GLP	:	Yes
Test substance	:	Other TS: Iron dichloride, purity = 98 % Sigma-Aldrich Corporation, Lot. No. 23828CB
Test conditions	:	- *Test Organisms* Age: 4 months Length: 2.9 ± 0.1 cm Weight: 0.22 ± 0.03 g Loading: 5.0 L of the test solution in 8.7 L aquarium per 7 fish Pretreatment: fish were acclimated for 7 days before test. No food was fed before 1 day and during test. - *Test Conditions* Dilution water source: Tap water passed through activated carbon and membrane filter (1 μm), hardness: 34 mg/L as $CaCO_3$, alkalinity: 24 mg/L as $CaCO_3$, Water chemistry: DO: 65 – 97 %, pH: 3.50 – 7.42 Temperature: 23.7 ± 0.3 °C Light: 890 – 1,030 Lux, Light periodicity: 16/8 (light/dark) A group of 7 fish was used without duplication.
Remarks	:	Iron dichloride is freely soluble in aqueous media. When ferrous ion reacts with hydroxide ions in water, solid ferrous hydroxide compounds such as $Fe(OH)_2(s)$ are formed. $Fe(OH)_2(s)$ reacts with oxygen and it changes to $4Fe(OH)_3(s)$. This reaction can be written as follows. $FeCl_2 + 2H_2O \rightarrow Fe(OH)_2 + 2HCl$ $Fe^{2+}(aq) + 2OH^-(aq) \rightarrow Fe(OH)_2(s)$ (green) $4Fe(OH)_2(s)$ (green) + $O_2(g) + 2H_2O \rightarrow 4Fe(OH)_3(s)$ (red-brown) These reactions usually occur slowly in pure water but several inorganic compounds in test media act as catalysts to process this reaction rapidly. 10 mg/L of iron dichloride solutions in distilled water and in culture media were tested for precipitation. Precipitation occurred in test media except distilled water, but the distilled water solution turned to pale yellow after several days. Iron dichloride exists as several forms as ferric ion, chloride ion and ferrous hydroxides in aqueous solution. Iron concentrations in the test solutions were analyzed with ICP-AES (measured substance was total iron (Fe) and measured total iron concentration was converted into iron dichloride concentration in the test solution). The results were obtained from the analyses of concentrations of iron dichloride in the test solution at 0, 48, 96 hours after the initiation of the study. Because of precipitation, the test solutions were mixed well before sampling. The measured concentrations were 70 – 140 % of nominal concentrations. The measured concentrations were used in this test instead of nominal concentrations.

Result : Measured concentrations were 11.7, 16.5, 24.0, 34.4, 62.6 and 99.2 mg/L (nominal concentrations at 10, 15, 24, 39, 63 and 100 mg/L). LC_{50} (96 hr) with 95 % confidence limit was 36.1 – 61.5 mg/L. LC_{50} value and 95 % confidence limit were calculated by Probit method (EPA/600/4-85/13, 1985).

Table. Cumulative mortality of *Oryzias latipes*

Nominal concentrations (mg/L)	Measured concentrations (mg/L)	Number of organisms tested	Cumulative number of dead fish			
			24 hrs	48 hrs	72 hrs	96 hrs
Control	Control	7	0	0	0	0
10	11.7	7	0	0	0	0
15	16.5	7	0	0	0	0
24	24.0	7	0	0	0	0
39	34.4	7	0	0	0	1
63	62.6	7	0	0	1	6
100	99.2	7	2	4	7	7

Table. pH of test solutions

Nominal concentrations (mg/L)	Measured concentrations (mg/L)	0 hr	24 hrs	48 hrs	72 hrs	96 hrs
Control	Control	7.42	7.15	7.17	7.25	7.27
10	11.7	6.75	7.00	7.07	7.09	7.17
15	16.5	6.58	6.82	6.97	7.01	7.07
24	24.0	6.36	6.49	6.65	6.67	6.80
39	34.4	6.11	5.89	5.34	4.11	4.19
63	62.6	5.93	5.61	4.72	3.65	3.50
100	99.2	5.71	5.53	4.21	-	-

Table. The results of acute toxicity test with pH adjustment

Nominal concentrations (mg/L)	pH	Number of organisms tested	Cumulative number of dead fish			
			24 hrs	48 hrs	72 hrs	96 hrs
100	3.50	5	5	5	5	5
100	4.00	5	0	0	0	0
100	4.50	5	0	0	0	0
100	5.00	5	0	0	0	0
100	5.50	5	0	0	0	0
100	6.00	5	0	0	0	0

When iron dichloride was dissolved in water, the test solution became acidic. So, a preliminary test was conducted to evaluate the effect of pH on fish. 100 mg/L of iron dichloride was dissolved and pH of the test solutions were adjusted to 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0. A group of 5 fish was used without duplication. The mortality was affected at pH at 3.5 only. Therefore, mortality increase in 62.6 mg/L at 96 hours was due to the low pH.

Reliability : (1) Reliable without restrictions
Flag : Critical study for SIDS endpoint

(22)

Type : Static
Species : *Tanichthys albonubes* (White cloud mountain minnow)
Exposure period : 48 hours
Unit : mg/L
Analytical monitoring : No

LC_{50}	:	> 100
Method	:	Kitamura. H., 1990
Year	:	1990
GLP	:	No data
Test substance	:	Other TS: Iron trichloride ($FeCl_3$, CAS No. 7758-08-0)
Remarks	:	- *Test Organisms* Length: 1.6 – 2.2 cm Loading: 7 fish in 100 mL of the test solution were prepared in a glass petridish of 12 cm in diameter. - *Test Conditions* For hardness of the solution, Potassium chloride(KCl), Magnesium sulfate ($MgSO_4$), Potassium sulfate (K_2SO_4) and Sodium bicarbonate($NaHCO_3$) were added. The hardness was adjusted to 30 ppm, 100 ppm, 200 ppm and 400 ppm. Dilution water was used as hardness of 0 ppm. Water chemistry: No data Temperature: 25 ± 1 °C A group of 7 fish was used without duplication. LC_{50} was calculated by Doudoroff. P. *et al*, 1951.
Result	:	

Table. The toxicity of the Iron trichloride to the *Tanichthys albonubes*

Toxicant	Chemicals	48 hrs LC_{50} Hardness (ppm)				
		0	30	100	200	400
Fe^{3+}	$FeCl_3$	> 100	-	> 100	-	> 100

Reliability	:	(3) Not reliable

(23)

Type	:	Static
Species	:	*Salmo gairdneri* (Rainbow trout)
Exposure period	:	No data
Unit	:	mg/L
Analytical monitoring	:	Yes
Method	:	R. Billard and P. Roubaud, 1985
Year		No data
GLP	:	No data
Test substance	:	Other TS: Iron dichloride tetrahydrate ($FeCl_2$•$4H_2O$, Prolabo CAS No. 13478-10-9)
Test conditions	:	- *Test Organisms* The gametes were taken from the stock of rainbow trout brood at the laboratory at Jouy-en-Josa between November and January during the period of ovulation and sperminogenesis. Female brood were examined at least once a week for ovulation. Ova from several females were pooled before the experiment. - *Test Conditions* Water temperature: between 8 and 12 °C
Remarks	:	The gametes were exposed independently for 40 min to iron dichloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, Prolabo) added into diluents. The ovum diluent (insemination diluent, ID) was a NaCl solution (osmotic pressure: 250 mOsm kg^{-1}, pH 9.0, Tris 20 mM, glycine 50 mM). The spermatozoa were diluted at 10^{-1} and 10^{-2} of ID with 30mM K^+ added in which they were immobile (a conservation diluent). Insemination was carried out after the gametes had been washed. After dilution, solubility of the test substance was measured by flame spectrophotometry after filtering. Ova and spermatozoa were mixed together in ID containing the test

chemical (insemination test). To test fertilization, the gametes were mixed with ID (about 150 – 200 ova + 10 mL of sperm: dilution of 10^{-3}) and left to stand for 15 min. The ova were rinsed and incubated at 10 ±1 °C for 10 days. The eggs were fixed in Stockard's solution and the percentage of eyed-eggs was an approximate fertilization rate. Tests were done in duplicate. Two fertilization percentages were compared using the X^2 test (controls vs experimental).

Result : Concentration of 0.005, 0.08, 0.73 and 1.13 mg/L were found in solutions for 1, 5, 30 and 100mg/L respectively.
Iron dichloride tetrahydrate in solution had a toxic effect on spermatozoa from concentrations of less than 0.005 mg/L at a dilution of 10^{-2} (ID) and from 0.73 mg/L at a dilution of 10^{-1} (ID). A similar sensitivity was found for ova (from 0.73 mg/L) and a higher sensitivity for fertilization (from 0.08 mg/L).

Reliability : (2) Reliable with restrictions

Flag : Critical study for SIDS endpoint

(24)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

A. Daphnia

Type	:	Static
Species	:	*Daphnia magna*
Exposure period	:	48 hours
Unit	:	mg/L
EC_{50}	:	19 mg/L
Analytical monitoring	:	Yes
Method	:	OECD TG 202, "*Daphnia sp*., Acute Immobilisation Test and Reproduction Test"
Year	:	2004
GLP	:	Yes
Test substance	:	Other TS: Iron dichloride, purity = 98 % Sigma-Aldrich Corporation, Lot. No. 23828CB
Test conditions	:	- *Test Organisms* Age: juveniles within 24 hours old. Supplier: GSF Institute of Ecological Chemistry, Germany - *Test Conditions* Dilution water source: OECD M4 medium, hardness: 247 mg/L as $CaCO_3$, alkalinity: 41 mg/L as $CaCO_3$ Water chemistry: DO: 84 – 99 %, pH: 5.92 – 8.41 Temperature: 21 ±0.5°C Light: 620 – 630 Lux, Light periodicity: 16/8 (light/dark) 3 replicates per 10 organisms were used.

Remarks : Iron dichloride is freely soluble in aqueous media. When ferrous ion reacts with hydroxide ions in water, solid ferrous hydroxide compounds such as $Fe(OH)_2(s)$ are formed. $Fe(OH)_2(s)$ reacts with oxygen and it changes to $4Fe(OH)_3(s)$. This reaction can be written as follows.

$FeCl_2 + 2H_2O \rightarrow Fe(OH)_2 + 2HCl$
$Fe^{2+}(aq) + 2OH^-(aq) \rightarrow Fe(OH)_2(s)$ (green)
$4Fe(OH)_2(s)$ (green) $+ O_2(g) + 2H_2O \rightarrow 4Fe(OH)_3(s)$ (red-brown)

These reactions usually occur slowly in pure water but several inorganic compounds in test media act as catalysts to process this reaction rapidly. 10 mg/L of iron dichloride solutions in distilled water and culture media were tested for precipitation. Precipitation occurred in test media except distilled water, but the distilled water solution turned to pale yellow after several days. Iron dichloride exists as several forms as ferric ion, chloride ion and ferrous hydroxides in aqueous solution. Iron concentrations in the test solutions were analysed with ICP-AES (measured substance was total iron (Fe) and measured total iron concentration was converted into iron dichloride concentration in the test solution). The results were obtained from the analyses of iron dichloride concentrations in the test solution at 0 and 48 hours from initiation of the study. Because of precipitation, the test solutions were mixed well before sampling. The measured concentrations were 97 – 112 % of nominal concentrations.

Result : Measured concentrations were 3.93, 7.14, 14.0, 24.2, 44.7 and 83.6 mg/L (Nominal concentrations at 3, 6, 12, 23, 45 and 90 mg/L). EC_{50} value with 95 % confidence limit was 15 – 25 mg/L. EC_{50} value and 95 % confidence limit were calculated by Probit method (EPA/600/4-85/13, 1985).

Table. The results of cumulative immobilization data for *Daphnia magna*

Nominal concentrations (mg/L)	Measured concentrations (mg/L)	Number of organisms tested	Cumulative number of organisms immobilized	
			24 hrs	48 hrs
Control	Control	30	0	0
3	3.93	30	0	4
6	7.14	30	0	5
12	14.0	30	0	11
23	24.2	30	0	13
45	44.7	30	12	23
90	83.6	30	30	30

Table. pH of test condition

Nominal concentrations (mg/L)	Measured Concentrations (mg/L)	0 hr	48 hrs
Control	Control	8.41	7.93
3	3.93	7.78	7.98
6	7.14	7.50	7.98
12	14.0	7.09	7.93
23	24.2	6.68	7.79
45	44.7	6.24	7.30
90	83.6	5.92	-

: The pH of test solutions remained neutral due to the buffering action of M4 medium.

Reliability : (1) Reliable without restrictions
Flag : Critical study for SIDS endpoint

(25)

Type	:	Static
Species	:	*Daphnia magna*
Exposure period	:	64 hours
Unit	:	mg/L
EC_{50}	:	< 38
Analytical monitoring	:	No data
Method	:	Anderson, 1944 and 1946
Year	:	1948
GLP	:	No data
Test substance	:	Other TS: Iron dichloride ($FeCl_2$, CAS No. 7758-94-3)
Remarks	:	- *Test Organisms* Age: 4 ± 4 hr old - *Test Conditions* The pH values of all solutions above 63.4 mg/L were lower than 7. Since the toxicity threshold was below this value, the EC_{50} was not influenced by acidity of the solution. Dilution water source: Lake Erie water Additions of Iron dichloride to Lake Erie water caused precipitation.
Reliability	:	(3) Not reliable

(26)

Type	:	Static
Species	:	*Daphnia magna*
Exposure period	:	24 hours
Unit	:	mol/L
EC_{50}	:	No data
Analytical monitoring	:	No data
Method	:	D.I. Stom and L.D. Zubareva, 1994
Year	:	In various seasons between 1988 and 1991
GLP	:	No data
Test substance	:	Other TS: Iron dichloride ($FeCl_2$, CAS No. 7758-94-3)
Remarks	:	- *Test Organisms* *Daphnia* specimens were grown by the standard procedure (Stroganov, N.S. and L.V.Kolosova, 1971) and individuals 2 to 3 days old were used in the experiments. - *Test Conditions* Water of the bicarbonate type from Lake Baikal that had been passed through miller's gauze was used in both the control and the experiments. The temperature was maintained at room temperature (20 °C) and the pH of the solutions was monitored during the experiment. 6 to 9 replicates per 6 organisms were used and *daphnids* were exposed to 30 mL of solution. The results were analyzed statistically, using confidence levels of 0.95 or higher.
Result	:	At concentration of 10^{-4} mol/L, *daphnia* had a survival rate of only 8 ± 2 percent in 24 hours. At 10^{-3} and 10^{-2} mol/L, *daphnia* had 100 percent mortality in 24 hours and a concentration of 10^{-5} mol/L failed to have an effect on *daphnia*.
Reliability	:	(2) Reliable with restrictions

(27)

Type	:	Static
Species	:	*Epischura baicalensis*
Exposure period	:	24 hours
Unit	:	mol/L
EC_{50}	:	No data
Analytical monitoring	:	No data
Method	:	D.I. Stom and L.D. Zubareva, 1994
Year	:	In various seasons between 1988 and 1991
GLP	:	No data

Test substance	:	Other TS: Iron dichloride ($FeCl_2$, CAS No. 7758-94-3)
Remarks	:	- *Test Organisms* *Epischura* specimens in copepodite stages IV-V were collected from Lake Baikal. - *Test Conditions* Water of the bicarbonate type from Lake Baikal that had been passed through miller's gauze was used in both the control and the experiments. The experiments with *Epischura* were performed in a temperature-controlled chamber at about 5 °C and the pH of the solutions was monitored during the experiment. 6 to 9 replicates per 20 organisms were used and *Epischura* were exposed to 30 mL of solution. The results were analyzed statistically, using confidence levels of 0.95 or higher.
Result	:	At concentrations of 10^{-4} mol/L, 10^{-3} and 10^{-2} mol/L, *Epischura* had 100 percent mortality in 24 hours and a concentration of 10^{-5} mol/L failed to have an effect on *Epischura*.
Reliabilities	:	(2) Reliable with restrictions

(27)

Type	:	Static
Species	:	*Daphnia magna*
Exposure period	:	48 hours
Unit	:	mg/L
EC_{50}	:	29.74
Analytical monitoring	:	No data
Method	:	Khangarot, B.S. and Ray, P.K., 1988
Year	:	1988
GLP	:	No data
Test substance	:	Other TS: Iron trichloride ($FeCl_3$, CAS No. 7758-08-0)
Remarks	:	- *Test Organisms* Age: within 12 hours old. Supplier: A natural pond situated at Gheru Campus of Industrial Toxicology Research Centre, Lucknow and a stock culture was made in tubewell water. - *Test Conditions* Dilution water source: filtered aerated tubewell water, hardness: 240 mg/L as $CaCO_3$, alkalinity: 400 mg/L as $CaCO_3$ Water chemistry: DO: 5.6 mg/L, pH: 7.6 Temperature: air temperature: 15 °C, water temperature: 13 °C 2 replicates per 10 organisms were used. Dose response curves were constructed by plotting percentage mortality as a function (EC_{50}) and associated 95 % confidence limits were determined by moving-average angle procedure.
Result	:	

Table. Toxicity of Iron(III) chloride ($FeCl_3$) by *Daphnia magna* screening

	Time	
	24 hrs	48 hrs
EC_{50} (mg/L)	74.41	29.74
95 % Confidence Limit (mg/L)	61.08 – 94.14	25.17 – 34.69

Reliability : (2) Reliable with restrictions

(28)

4.3 TOXICITY TO AQUATIC PLANTS

Algae

Species	:	Selenastrum capricornutum
Endpoint	:	Growth rate
Exposure period	:	72 hours
Unit	:	mg/L
E_gC_{50}	:	6.9
E_bC_{50}	:	3.8
$NOEC_r$	:	2.4
$NOEC_b$	:	1.1
$LOEC_r$		5.1
$LOEC_b$		2.4
Analytical monitoring	:	Yes
Method	:	OECD TG 201, "Alga, Growth Inhibition Test"
Year	:	2001
GLP	:	Yes
Test substance	:	Other TS: Iron dichloride (Aldrich Chemical Co., CAS No. 7758-94-3), purity = 99.99 %, Lot. No. – 02442PI
Test conditions	:	- Test organisms Laboratory culture: ATCC culture medium 625 Gorham's medium Strain No.: ATCC 22662 Method of cultivation: sterilization - Test conditions Temperature: 22 – 24 °C Dilution water source: OECD medium Water chemistry: pH 7.04 – 8.0 at the beginning and pH 4.74 – 7.64 at the end of tests. Light level: 7,940 – 8,318 Lux Initial cell density: 1 x 10^4 cells/mL Test design: 3 replicates
Remarks	:	Iron concentrations in the test solutions were analysed with ICP-AES (measured substance was total iron (Fe) and measured total iron concentration was converted into iron dichloride concentration in the test solution). The results obtained from the analyses of concentrations of iron dichloride in algae growth inhibition test solution at 0, 24, 48 and 72 hours from initiation of the study. Because of precipitation, the test solutions were mixed well before sampling. The measured concentrations were 75 – 85 % of nominal concentrations. So, measured concentration was used in this test instead of nominal concentration.
Result	:	Measured concentrations = 1.1, 2.4, 5.1, 12, 22 and 44 mg/L (Nominal concentrations at 3, 6, 13, 25, 50 and 100 mg/L) were studied. EC_{50}, value and NOECs were calculated by Comprehensive Toxicity Data Analysis, database Software (Version 5.0) and Dunnett's test, respectively.

Table. Cell density of *Selenastrum capricornutum* (ATCC 22662) during the test

Nominal Concentrations (mg/L)	Measured Concentrations (mg/L)	Cell density (x 10^4 cell/mL)			
		0 hr	24 hrs	48 hrs	72 hrs
Control	Control	1.1	2.1	24	190
3	1.1	0.92	2.5	22	130
6	2.4	1.4	2.5	26	95
13	5.1	1.1	1.4	22	120
25	12	0.84	0.55	11	1.9
50	22	0.67	0.21	0.64	0.34
100	44	0.79	0.083	0.34	0.083

Table. Percent inhibition of growth rates per concentration.

Nominal Concentrations (mg/L)	Measured Concentrations (mg/L)	Growth rates		
		Growth rate	Relative growth rates (%)	Relative inhibition (%)
Control	Control	0.072	-	-
3	1.1	0.068	95.5	4.5
6	2.4	0.065	91.1	8.9
13	5.1	0.058	81.0	19.0
25	12	0.012	16.3	83.7
50	22	-0.047	0	100
100	44	-0.088	0	100

Nominal Concentrations (mg/L)	Measured Concentrations (mg/L)	Areas under the curve		
		Areas under the curve	Relative growth rates (%)	Relative inhibition (%)
Control	Control	28,708,000	-	-
3	1.1	23,680,000	82.5	17.5
6	2.4	19,268,000	67.1	32.9
13	5.1	17,488,000	61.0	39.0
25	12	761,200	2.7	97.3
50	22	-154,796	0	100
100	44	-365,192	0	100

Table. pH of test solutions

Nominal Concentrations (mg/L)	Measured Concentrations (mg/L)	0 hr	72 hrs
Control	Control	7.81	7.61
3	1.1	7.72	7.44
6	2.1	7.69	7.64
13	5.1	7.24	7.51
25	12	8.00	6.86
50	22	7.50	5.48
100	44	7.04	4.74

Precipitation of test substance was observed at 12 – 44 mg/L. The condition of test solutions of 12, 22 and 44 mg/L became acidic after 72 hours. The acidic condition of test solutions affected the growth of algae.

Reliability : (1) Reliable without restrictions.
Flag : Critical study for SIDS endpoint

(29)

4.4 TOXICITY TO MICROORGANISMS

4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6 TERRESTRIAL ORGANISMS

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 AVIAN)

4.7 BIOLOGICAL EFFECTS MONITORING (INCLUDING BIOMAGNIFICATION)

4.8 BIOTRANSFORMATION AND KINETICS

Remarks : Photochemically formed Fe(II) is reoxidized by O_2. This oxidation is enhanced if the ferrous iron is adsorbed on mineral (or biological) surface. The reoxidation of Fe(II) produces a "$Fe(OH)_3$"(s) as a colloid or on the surface that is less polymeric and less crystalline than aged Fe(III) hydroxides, and thus more soluble and in faster eqilibrium with monomeric (inorganic) Fe(III) species, which may control iron uptake by phytoplankton.

Reliability : (4) Not assignable

(21)

4.9 ADDITIONAL REMARKS

Remarks : Several aquatic toxicity studies for iron salts are as follows;
Ferric chloride;
Brachydanio rerio: LC_0 (48 hr) = 92.8 mg/L
Poecillia reticulata: LC_{50} (48 hr) = 117 mg/L
Oryzias latipes: LC_{50} (48 hr) = 23 mg/L
Daphnia magna: LC_{50} (48 hr) = 21 – 30 mg/L
Chlorella vulgaris: NOEC (120 day) = 2.7 mg/L
LOEC (120 day) = 5.45 mg/L
Ferric sulphate;
Oncorhynchus mykiss: LC_{50} (96 hr) = 45.8 mg/L
(> 100 mg/L with pH adjustment)
Daphnia magna: LC_{50} (96 hr) = 11.48 mg/L Fe
Scenedesmus sp.: NOEC (7 day) = 5.6 mg/L
Ferrous sulphate;
Oncorhynchus mykiss: LC_{50} (96 hr) = 45 mg/L
(pH 6.9 based on nominal addition)
Oncorhynchus mykiss: LC_{50} (96 hr) = 10.8 mg/L
(pH 6.9 based on analysised Fe^{2+} in solution at end point)
Daphnia magna: LC_{50} (48 hr) = 19.4 mg/L
Daphnia magna: LC_{50} (48 hr) = 35.1 mg/L
Chlorella vulgaris: NOEL (30 day) = 700 mg/L

Reliability : (4) Not assignable

(14)

5. TOXICITY

5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Species/Strain	:	Rat (Sprague-Dawley)
Sex	:	Female
Number of animals	:	9 animals
Vehicle	:	Corn oil
Value	:	300 mg/kg for 1st and 2nd steps, and 2,000 mg/kg for limit dose
Route of administration	:	Oral (gavage)
Method	:	OECD Test Guideline 423 annex 2c
Year	:	2004
Method remarks	:	- Preparation of test substance Formulations were prepared in corn oil (CS149: Sigma, Lot No: 122K0131). The test article was weighed, dissolved in an appropriate volume of vehicle. The solution was diluted with the vehicle to the required volume and concentration and subsequently mixed using a vortex mixer and homogenizer. - Treatment procedure The starting dose of 300 mg/kg body weight as the 1st step was selected. Three additional rats were tested with the same dose as the 2nd step since there was no mortality in the first group. The 3rd step was the limiting dose of 2,000 mg/kg body weight. - Administration Animals were fasted overnight prior to dosing and the body weights were measured after the fasting. Formulations were administered by single oral gavage using a disposable flexible needled attached to a disposable plastic syringe at a dose volume of 10 ml/kg body weight. After the test article has been administered, food was withheld for 3 to 4 hours. - Clinical observations Cages of rats were checked at least once a day for any mortality. Animals were individually observed hourly during the first 4 hours after dosing with special attention, and daily for a total of 14 days. The nature and severity of the clinical signs and time were recorded at each observation. - Body weights The body weight of each rat was recorded on day 1 (prior dosing), day 8 and day 15 (prior to necropsy). Individual body weight changes were calculated. - Necropsy All survived animals were killed on day 15 by carbon dioxide asphyxation and subjected to gross necropsy consisted of opening the thoracic and abdominal cavities. Necropsy was performed on the dead animals immediately, when they were found. All gross pathological changes were recorded for each animal.
Year	:	2004
GLP	:	Yes
Test substance	:	Other TS: Iron dichloride, purity = 98 %, Sigma-Aldrich, 37,287-0 Lot No. – 23828CB
Test conditions	:	- Age of test animals: 9 – 10 weeks - Body weights of test animals during the study: 156.2 – 171.9 g

- Frequency of treatment: single treatment
- Post dose observation period: 14 days

Results : - Mortality
All animals received 2,000 mg/kg body weight died after one hour and one animal received 300 mg/kg body weight was found dead on day 2.
- Clinical Signs
Hypoactivity, piloerection, prone position, reddening and edema on ears, fore-legs and hind-legs, dyspnea, incomplete eyelid opening and hypothermia were observed in the three dead animals of 2,000 mg/kg and in one dead animall of 300 mg/kg group. These signs were considered to be distressful and painful symptoms caused by acute systemic toxicity of the test article. At 300 mg/kg body weight, all animals showed hypoactivity and piloerection on day 1. Some animals showed soft stool on day 2, but these symtoms were recovered.
- Body weights
All survived rats gained normal body weight throughout the study.
- Gross pathology
No abnormalities were observed in all survived animals. At 2,000 mg/kg body weight, nasal discharge (reddish or clear) was observed in all animals externally. Hemorrhage on lymphatic nodes, stomach and intestine in all animals and hemorrhage on thymus in one animal were observed. One animal showed hypertrophy of pancreas and other one animal showed the hypertrophy of spleen.
At 300 mg/kg body weight, hemorrhage on lymphatic nodes, and intestine were observed in the dead animal. And there was no abnormality by the macroscopic examination of survived animals after the study termination on day 15.

Table. Mortality and clinical signs

Group	Dose (mg/kg)	Animal No.	Clinical signs observed in days after treatment						Mortality (%)
			Hours on day 1 after treatment				Day 2	Day 3-15	
			1	2	3	4			
1	300	F1	H, P	H, P	H, P	H	N	N	0/3 (0)
		F2	H, P	H, P	H, P	H	Ss	N	
		F3	H, P, S	H, P	H, P	H	Ss	N	
2	300	F4	H, P	H, P	H, P	H	N	N	1/3 (33)
		F5	P	H, P	H, P	H	Ss	N	
		F6	H. Le. P. Pp	H. Le. P. Pp	Dy. H. Le. P. Pp	Ht. H. Le. P. Pa	FD	-	
3	2000	F7	H. P. Pp, †		FD		-	-	3/3 (100)
		F8	H. P. Pp, Di, †		FD		-	-	
		F9	H. P. Pp, †		FD		-	-	

Dy: Dyspnea; FD: Found Dead; H: Hypoactivity; Ht: Hypothermia; Ie: Incomplete eyelid opening; N: Normal; P: Piloerection; Pa: Pale; Pp: Prone position; S: Salivation; Ss: Soft stool; Di: Diarrhea; †: Reddish change and edema on ears, fore-legs and hind-legs.

Table. Summary of group mean body weights

Group	Dose (mg/kg)	Mean body weight ± S.D. (g)		
		Day 1	Day 8	Day 15
1	300	163.9 ± 6.9	206.6 ± 6.3	228.7 ± 12.5
2*	300	161.6 ± 1.7	207.4 ± 7.4	226.7 ± 6.6
3**	2,000	162.5 ± 6.1	-	-

* One animal was found dead 2 days after administration.
** All animals were found dead 2 days after administration.

Table. Gross necropsy findings (Group summary)

Group			1	2	3
Dose (mg/kg)			300	300	2,000
Number of animals examined at terminal kill			3	3	3
External finding	No gross finding		3	3	
	Reddish nasal discharge				1
	Clear nasal discharge				2
Internal finding	No gross finding		3	2	
	Hemorrhage	Intestine		1	3
		Lymphatic node		1	3
		Ovaries		1	
		Pancreas			2
		Stomach			1
		Thymus			1
	Hypertrophy	Pancreas			1
		Spleen			1

Conclusions : All animals received 2,000 mg/kg body weight and one animal received 300 mg/kg body weight was found dead. The acute lethal oral dose (LD_{50} cut-off values) to rats of the test article, Iron dichloride was estimated to be between 300 and 2,000 mg/kg body weight under the test conditions.
Reliability : (1) Reliable without restrictions
Flag : Critical study for SIDS endpoint
Reference : (30)

Type : LD_{50}
Species/Strain : Rat
Value : 600 mg/kg
Remarks : There are no information about tested animals, experimental conditions and tested chemical.
Year : 1955
Test substance : Other TS; Iron dichloride
Reliability : (4) Not assignable
Reference : (31)

Type : LD_{50}
Species/Strain : Mouse
Value : 895 mg/kg
Remarks : There is no information about tested animals, experimental conditions and tested chemical.
Details of toxic effects not reported other than lethal dose value.
Year : 1976
Test substance : Other; Iron tridichloride (7705-08-0)
Reliability : (4) Not assignable
Reference : (32)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}
Species/strain : Rat (Sprague-Dawley)
Sex : Male and Female
No of animals : Male 5 and Female 5
Route of Administration : Test article was applied directly to the skin after clipping fur.
Exposure time : 24 hours

Value	:	2,000 mg/kg
Method	:	OECD Test Guideline 402
Method remarks	:	- Preparation of test substance In this study, test article was weighed and wetted with corn oil (CS149, Sigma, Lot No. 122K0131). - Treatment procedure At first, the limit tests at one dose level of 2,000 mg/kg body weight were performed. - Administration of the test article Animals were clipped their fur on dorsal part approximately 10 % of the total body surface area one day before dosing. The test article was applied uniformly and held in contact with the skin with a porous gauze dressing and non-irritating tape for 24-hour exposure period. At the end of the exposure period, residual test article was removed using distilled water. - Clinical observations Cages of rats were checked at least once a day for any mortality. Animals were individually observed hourly during the first 4 hours after dosing with special attention, and daily for a total of 14 days. The nature and severity of the clinical signs and time were recorded at each observation. - Body weights The body weight of each rat was recorded on day 1 (prior to dosing), day 8 and day 15 (prior to necropsy). Individual body weight changes were calculated. - Necropsy All animals were killed on Day 15 by carbon dioxide asphyxiation and subjected to gross necropsy consisted of opening the thoracic and abnormal cavities. All gross pathological changes were recorded for each animal.
Year	:	2004
GLP	:	Yes
Test substance	:	Other TS: Iron dichloride, purity = 98 %, Sigma-Aldrich, Lot No. – 23828CB
Test conditions	:	- Age of test animals: 8 weeks - Body weight of test animals during the study: 256.9 – 279.7 g for males and 223.8 – 233.5 g for females - Dose per time period: single treatment - Post dose observation period: 14 days
Results	:	- Mortality and clinical signs During the study, there was no unscheduled death. Yellowish-brown change on the skin of applied site was observed in all treated animals from day 2 but this sign was recorvered on day 15 except 3 animals. 2 males and 4 females showed the reddish nasal discharge on day 2. - Body weights All rats gained normal body weight throughout the study. - Gross pathology Internally, no abnormalities were observed in all animals by microscopic examination. On applocation sites, scars were observed in one male and one female.

Table. Mortality and clinical signs

Group	Sex	Animal No.	Clinical signs observed in days after treatment														Mortality (%)
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	male	M1	N	Ya	Ya	Ya	†	N	N	N	N	N	N	N	N	N	N	0/5 (0)
		M2	N	Ya	Ya	Ya	Ya	†	†	†	†	†	†	†	N	N	N	
		M3	N	Ya, Nd	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	†	†	N	N	N	
		M4	N	Ya, Nd	Ya	Ya	Ya	†	†	†	†	†	†	†	N	N	N	
		M5	N	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	†	†	†	N	N	N	
	female	F6	N	Ya, Nd	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	†	†	†	†	0/5 (33)
		F7	N	Ya, Nd	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	†	†	†	†	
		F8	N	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	†	†	†	†	
		F9	N	Ya, Nd	Ya	Ya	Ya	Ya	Ya	†	†	†	†	†	N	N	N	
		F10	N	Ya, Nd	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	Ya	†	†	N	N	

Group 1: Treatment (2,000 mg/kg), N: Normal; Nd: Red nasal dischage; Ya: Yellowish-brown change on application sites; †: Slight yellowish-brown change on application sites.

Table. Summary of group mean body weights

Sex	Animal number	Body weight (g)		
		Day1	Day8	Day15
Male	M1	275.1	312.8	360.1
	M2	279.7	319.7	379.1
	M3	266.2	286.6	329.7
	M4	265.8	310.7	369.2
	M5	256.9	293.9	331.7
	Mean ± S.D.	268.7 ± 8.89	304.7 ± 13.88	354.0 ± 22.28
female	F6	225.1	243.2	253.3
	F7	232.3	241.6	250.0
	F8	226.9	242.9	260.6
	F9	233.5	245.5	269.1
	F10	223.8	231.2	252.2
	Mean ± S.D.	228.3 ± 4.34	240.9 ± 5.59	257.0 ± 7.83

Table. Gross necropsy findings (Group summary)

Dose (mg/kg/day)		2,000	
Sex		Male	Female
Number of animals examined		5	5
External finding	No gross finding	4	4
	Scar on applied site	1	1
Internal finding	No gross finding	5	5

Conclusions : The acute lethal dose (LD_{50} values) of iron dichloride to rats by single dermal administration was considered to be greater than 2,000 mg/kg body weight under the conditions of this study.

Remarks	:	
Reliability	:	(1) Reliable without restrictions
Flag	:	Critical study for SIDS endpoint
Reference	:	(33)

5.1.4 ACUTE TOXICITY BY OTHER ROUTES OF ADMINISTRATION

Type	:	LD_{50}
Species/strain	:	Mouse
Sex	:	No data
Number of animals	:	8 – 16
Vehicle	:	Water
Value	:	59 mg/kg
Route of administration	:	Intraperitoneal
Method	:	Other
Year	:	1962
GLP	:	No
Test substance	:	Other TS: Iron dichloride, 7758-94-3 There are no information of source, purity and stability of chemical.
Remarks	:	There are no information about test methods and test conditions.
Results	:	LD_1: 33.2 mg/kg, LD_{50}: 59.0 mg/kg, LD_{99}: 102.0 mg/kg There are no information about mortality and clinical signs. The probability limit (a = 0.05) was obtained by the method of Litchfield and Wilcoxon
Test conditions	:	Test organisms: white mouse Number of Animals: 8 – 16 Administration: 1 ml/20 g of mice Route of Exposure: Intraperitoneal injection Dose Levels: 0.06 – 0.001 molar
Reliability	:	(3) Not reliable
Reference	:	(34)

Type	:	LD_{50}
Species/strain	:	Mouse
Sex	:	No data
Number of animals	:	8 – 16
Vehicle	:	Water
Value	:	92.5 mg/kg
Route of administration	:	Intraperitoneal
Method	:	Other
Year	:	1962
GLP	:	No
Test substance	:	Other : Iron dichloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), 13478-10-9
Remarks	:	There are no information about test methods and test conditions.
Results	:	LD_1: 52.0 mg/kg, LD_{50}: 92.5 mg/kg, LD_{99}: 160.0 mg/kg There are no information about mortality and clinical signs. The probability limit (a = 0.05) was obtained by the method of Litchfield and Wilcoxon.
Test conditions	:	Test organisms: white mouse Number of Animals: 8 – 16 Administration: 1 ml/20 g of mice Route of Exposure: Intraperitoneal injection Dose Levels: 0.06 – 0.001 molar
Reliability	:	(3) Not reliable
Reference	:	(34)

Type	:	LD_{50}
Species/strain	:	Mouse
Value	:	58 mg/kg
Route of administration	:	Intravenous
Method	:	Other
Year	:	1967
GLP	:	No
Test substance	:	Other: Iron trichloride ($FeCl_3$), 7705-08-0
Remarks	:	There are no information about test methods and test conditions.
Reliability	:	(4) Not assignable
Reference	:	(35)

Type	:	LD_{50}
Species/strain	:	Mouse
Value	:	260 mg/kg
Route of administration	:	Intraperitoneal
Method	:	Other
Year	:	1966
GLP	:	No
Test substance	:	Other: Iron trichloride hexahydrate, 10025-77-1
Remarks	:	There are no information about test methods and test conditions.
Reliability	:	(4) Not assignable
Reference	:	(36)

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

Test type	:	*In vivo*
Species/strain	:	Rabbit (New Zealand White)
Sex	:	Male
Number of animals	:	3 animals
Vehicle	:	Not used
Value	:	0.5 g
Route of administration	:	Fur removed skin (2 x 3 cm)
Results	:	Weak irritant
Method	:	OECD TG 404 (2002) “Acute Dermal Irritation/Corrosion”
Method remarks	:	- Preparation of test substance Iron dichloride was ground to fine powder and applied to the skin patch and then moistened with water. - Treatment procedure For the initial test, three test pathches (0.5 g of test article/patch) were applied sequentially on one animal. The first patch was removed after 3 minutes and 2nd patch was applied on other site for one hour. The third patch was applied on another site for 4 hours. Then, conformatory test was conducted in 2 additional animals. - Administration of the test article 500 mg of test material wetted with sterile distilled water was applied to the abraded sites on the shaved backs (2 x 3 cm) of rabbits. The test article was applied uniformly and held in contact with the skin with a porous gauze dressing and non-irritating tape. At the end of the exposure period, residual test article was washed off gently with sterilized saline solution.

- Clinical observations and skin reactions
The skin reaction signs of erythma and oedema on application sites were examined at 1, 24, 48 and 72 hours after the patches were removed. The grades of skin reaction was scored according to the scoring method suggested in OECD TG 404. Mortality, clinical signs and body weight were also investigated.

Year : 2004
GLP : Yes
Test substance : Other TS: Iron dichloride, purity = 98 %, Sigma-Aldrich, Lot No. – 23828CB
Test conditions : Test organisms: rabbits
- Age: 3 to 4 months old
- Numbet of animals: 3 animals
- Weight: 2.3 – 2.7 kg
- Scoring method: (OECD TG 404 – Grading of skin reaction)

Results : - Mortality and clinical signs
During the study, there was no animal death. No specific clinical signs were observed except yellowish brown colorations on the application sites.
- Body weights
All tested animals gained normal body weights.
- Local irritancy
The weak edema was observed on the application sites. However, this sign was recovered within 1 day after the patch was removed.

Table. Mortality and clinical signs

Group	Animal No.	Days after treatment					Mortality (%)
		1	2	3	7	14	
1	M1	0	0	0	0	0	0
	M2	0	0	0	0	0	
	M3	0	0	0	0	0	

Table. Summary of body weight changes

Group	Animal No.	Body weight changes (g)	
		Day 0	Day14
1	M1	2,719.7	3,024.0
	M2	2,308.3	2,830.3
	M3	2,516.6	2,539.7
	Mean ± S.D.	2,514.87 ± 167.96	2,798 ± 199.03

Table. Grading of skin irritation

Type of skin reaction	Observation time	Score		
		Initial test	Confirm test	
	Animal No	M1	M2	M3
Erythema & Eschar	3 min	0	-*	-
	1 hr	0	0	0
	4 hr	0	-	-
	24 hr	0	0	0
	48 hr	0	0	0
	72 hr	0	0	0
	7 day	0	0	0
	14 day	0	0	0
Oedema	3 min	0	-	-
	1 hr	1	1	1
	4 hr	1	-	-

	24 hr	0	0	0
	48 hr	0	0	0
	72 hr	0	0	0
	7 day	0	0	0
	14 day	0	0	0

* The clinical observations were not conducted at 3 min and 4 hour after application in confirm tests.

Conclusions : Iron dichloride is a weak irritant inducing grade 1 of oedema on application site.
Reliability : (1) Reliable without restrictions
Flag : Critical study for SIDS endpoint
Reference : (37)

5.2.2 EYE IRRITATION/CORROSION

Test type : *In vivo*
Species/strain : Rabbit (New Zealand White)
Sex : Male
Number of animals : 3 animals
Vehicle : Not used
Value : 0.1 g
Route of administration : Eye sac
Results : Corrosive irritant
Method : OECD TG 405 (2002) “Acute Eye Irritation/Corrosion”
Method remarks : - Preparation of test substance
Iron dichloride was ground to fine powder and then applied to the eye sac.
- Treatment procedure
0.1 g of preparated iron dichloride was applied into the right. After 1 hour, the remaining test compound was removed by gentle washing with sterilized saline solution. The clinical signs were observed at 1, 24, 48 and 72 hours. After initial test, confirmatory test was performed with 2 additional animals.
- Clinical observations
During the test, changes in clinical signs, toxic effects and mortality of tested animals were recorcded. The pathological effects on eyes were examined under the microscope. To determine reversibility of the lesions, the clinical signs were observed on 7th, 14th, and 21th days.
- Body weights
Body weights of tested animals were measured at the initiation and termination of test.
- Evaluation and determination of irritancy
The grade of ocular response was scored according to TG405.
- Histopathology
After termination of test, the eye ball, conjunctiva and eyelids were fixed in Davidson solution and 10 % formalin solution for histopathological examinations.
Year : 2004
GLP : Yes
Test substance : Other TS: Iron dichloride, purity = 98 %, Sigma-Aldrich, Lot No. – 23828CB
Test conditions : Test organism: rabbits
- Age: 3 to 4 months old
- Numbet of animals: 3 animals
- Weight: 2.2 – 2.4 kg
- Scoring method: OECD TG 405 – Grading of ocular lesions

Results : - Mortality and clinical signs
During the study, there was no animal death.
Motility was reduced in all tested animals and rubbing eye with a forefoot was observed in 2 animals.
- Body weight
All tested animals showed normal body weight increase.
- Clinical observation of the eyes
The irritant response was observed in all animals within 1 hour. The cornea, iris and conjunctiva were affected by test compounds. The level 3 of opacity area (greater than three-quarters) were seen in cornea within 1 hour after treatment. The degree of opacity was increased to the level 4 in 3 days and the iris was not discernible through the opacity. These cornea lesions were not recovered within the test periods. Iris was also affected by iron dichloride. Hyperaemia and destructed light-reactivity were seen at 2 days after treatment. Severe redness, edema and swelling were also observed in conjunctiva within 1 hour. This swelling effect caused partial eversion of lids and more than half of lids were closed. Although the pathological legions were recovered partially, the eyelids were transformed abnormally.
- Histopathology
There were lymphocyte infiltration in the conjunctiva and severe granulomatous legions in cornea. A purulent inflammation and corneitis were also observed in stroma of cornea. The clinical signs of iris shown in the early stage were recovered within 21 days since there was no histopathological changes.

Table. Mortality and clinical signs

Animal No.	Days after treatment							Mortality (%)
	1	2	3	4	7	14	21	
M1	0	0	0	0	0	0	0	0
M2	0	0	0	0	0	0	0	
M3	0	0	0	0	0	0	0	

Table. Summary of body weight changes

Group	Animal number	Body weight changes (g)	
		Day 0	Day 21
1	M1	2,378.9	2,982.0
	M2	2,398.6	2,959.7
	M3	2,376.4	2,977.7
	Mean ± S.D.	2,384.63 ± 12.16	2,973.13 ± 11.83

Table. Grading of eye irritation

Type of eye response		Observation time	Score		
			Initial test		Confirm test
			M1	M2	M3
Cornea	Degree of opacity	1 hr	1	1	1
		24 hr	2	2	3
		48 hr	2	3	4
		72 hr	3	4	4
		7 day	4	4	4
		14 day	4	4	4
		21 Day	3	4	4
	Diffuse areas of opacity	1 hr	3	3	3
		24 hr	3	3	3
		48 hr	3	3	3
		72 hr	3	3	3

		7 day	3	3	3
		14 day	3	3	3
		21 Day	2	3	3
Iris		1 hr	0	0	0
		24 hr	1	1	1
		48 hr	1	2	2
		72 hr	2	2	2
		7 day	2	2	2
		14 day	2	2	2
		21 Day	2	2	2
Conjunctiva	Redness	1 hr	1	2	2
		24 hr	2	2	2
		48 hr	2	3	3
		72 hr	3	2	3
		7 day	2	2	2
		14 day	1	2	2
		21 Day	1	2	2
	Edema	1 hr	2	2	4
		24 hr	2	4	4
		48 hr	3	4	4
		72 hr	3	3	4
		7 day	2	3	4
		14 day	2	2	3
		21 Day	2	2	3

* Diffuse areas of apacity: 0 (one-quarter (or loss), not zero), 1 (Greater than one-quarter, but less than half), 2 (Greater than half, but less than three-quarters), 3 (Greater than three-quarters, up to whole area).

Conclusions : Iron dichloride is a corrosive irritant on eyes.
Reliability : (1) Reliable without restrictions
Flag : Critical study for SIDS endpoint
Reference : (38)

Type : Skin and eye irritation
Remarks : Range of toxicity
Direct contact: Slight eye, skin, and respiratory irritant
General sensation: Skin and eye contact may produce severe irritation and burns. Inhalation of dust may cause coughing, choking and respiratory difficulty.
Conclusions : Iron dichloride causes an irritation on skin by direct contact
Reliability : (4) Not Assignable
Reference : (39)

Type : Skin and eye irritation
Remarks : Hazard Summary
- Iron dichloride can affect you when breathed in.
- Iron dichloride is a corrosive chemical and contact can irritate and burn the eyes and skin.
- Prolonged contact may discolor the eyes.
- Breathing Iron dichloride can irritate the nose and throat.
- Repeated or high level exposures may lead to too much iron build-up in the body causing nausea, stomach pain and vomiting.
- Iron dichloride may damage the liver.

Conclusions : Iron dichloride can irritate and burn the skin.
Reliability : (4) Not Assignable
Reference : (40)

Type	:	Skin and eye irritation
Remarks	:	Health Hazards Symptoms Following Exposure: Inhalation of dust irritates nose and throat. Ingestion causes irritation of mouth and stomach. Dust irritates eyes and may cause skin irritation on prolonged contact.
Conclusions	:	Iron dichloride may cause skin irriatation on prolonged contact.
Reliability	:	(4) Not Assignable
Reference	:	(41)

5.3 SKIN SENSITISATION

5.4 REPEATED DOSE TOXICITY

Species/strains	:	Rat (Sprague-Dawley)
Sex	:	Male/Female
Route of administration	:	Oral (Gavage)
Method	:	OECD TG 422 “Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test”
Year	:	2004
GLP	:	Yes
Test substance	:	Other TS: Iron dichloride purity = 98 %, Sigma-Aldrich Corporation, LOT No. – 14330TA
Dose levels	:	0, 125, 250 and 500 mg/kg b.w./day
Exposure period	:	42 days for male animals and 42 to 54 days for female animals
Frequency of treatment	:	Daily
Control groups	:	Yes (Concurrent no treatment)
Post exposure observation period	:	2 weeks for recovery groups
Statistical methods	:	Homogeneity of variance was evaluated using Levene’s test in terms of body weight, food and water consumption, biochemical test of blood and organ weight. When the assumption of homogeneity of variance was met, ANOVA was used. If significant result was observed, Dunnett’s test was used. When the assumption of heterogeneity of variance was met, appropriate data transformation was carried out, then Levene’s test was performed on re-transformed data. If significant result was observed, Dunnett’s test was used.

Test conditions : Test organism
- Sex: male/female
- Age of animals at study: 8 weeks old for males and females
- Weight during the study: 269.23 – 302.18 g for males and 191.34 – 221.60 g for females
- Number of test animals: 70 animals for each sex
- 10 animals (5 males + 5 females) from G1 (0 mg/kg b.w./day) and from G4 (500 mg/kg b.w./day) groups were allocated as recovery groups.

Observation of F0
- *Clinical observations performed and frequency*: Clinical symptoms were observed once a day and once a week in detail. The death rate was observed twice a day. The body weights were measured once a week and right before the necropsy except mating period, but for pregnant females, it was measured on day 0, 7, 14, 20 of gestation period, date of delivery, and 4 days after the delivery. Consumption rate of fodder was measured once a week except mating period.
- *Test for sensory organ*: Five males and five females were randomly selected from each test group. Both auricle reflex test and corneal reflex test were performed before necropsy.
- *Motor function test*: Five males and five females were randomly selected from each test group for traction test. This test was performed before necropsy.
- *Urinalysis*: Five males and five females were randomly selected from each test group. Following seven items were tested; color, specific gravity, pH, glucose, protein, leukocyte, erythrocyte.

- *Hematological test*: Randomly selected 5 male and 5 female animals from each test group were fasted for 18 hrs before necropsy. Animals were anesthetized with ether and the abdomen was cut open to collect blood. One ml of gathered blood was put in CBS bottle with EDTA then following 13 items were measured using erythrocyte analyzer; total erythrocyte count (RBC), hemoglobin concentration (HEG), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), total leucocyte count (WBC), platelet (PLT), neutrophils (NEU), eosinophils (EOS), basophils (BASO), lymphocytes (LYM), and monocytes (Mono). After blood collection, sera were separated using a centrifuge to measure prothrombin time (PT) and activated partial thromboplastin time (APTT). Metheamoglobin (MH) was also analyzed.
- *Biochemical test:* Following 14 items were measured: alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, total protein, albumin, sodium, potassium, triglycerides, glucose, phosphorus, calcium and cholinesterase. Cholinesterase II actibity was measured with S-butyrylthilcholine iodide as a substrate.

Organs examined at necropsy:
- *Organ weight*: testes, epididymider (all males), liver, kidney, adrenal, thymus, spleen, brain and heart (5 male and 5 female animals from each test group).
- *Fixation*: 21 tissues were preserved in 10 % buffered neutral formalin solution for histopathologic tests: brain, pituitary, spinal cord, heart, lung, trachea, stomach, ileum, liver, colon, spleen, thyroids, thymus, adrenals, kidneys, urinary bladder, sciatic nerve, bone marrow, uterus, ovaries and lymph node. Testes and epididymides were fixed in bouin's fixative.

NOAEL : 125 mg/kg b.w./day for male, 250 mg/kg b.w./day for female.

Results : Preliminary tests
All male rats in 1,000 mg/kg b.w./day treatment group were dead. For female rats, one rat was dead at the same dose level. Therefore, 500 mg/kg b.w./day was chosen as the maximum dosage.

Main test
Results for F0
- *Mortality*: No death was observed for male animals. Three female rats in 500 mg/kg b.w./day treatment group were found dead on the day 38, 46 and 51 of adminstration.
- *Clinical signs*:
Clinical signs such as blackish stool and salivation were observed in both the control and the treated groups. In the early stages of administration, cases of decrease in locomotion activity were found in 500 mg/kg b.w./day groups of both sexes, but these were recovered to normal states. The female rats were more sensitively affected than the male rats in locomotion activity decrease, paleness, emaciation and soiled perineal region. However, these symptoms were reversible within the test period.
- *Body weight*
The rate of body weight gain was significantly decreased in 250 and in 500 mg/kg b.w./day male groups. For females, there was no significant changes except on the day 7 of pre-mating period and the day 4 of lactation period. Further, no dose-dependent changes were shown.
- *Food consumption*: There was no significant difference between the control and the treated groups, and no dose-related change was observed in both sexes.
- *Water consumption*: In 500 mg/kg b.w./day treatment group, the amount of water consumption was increased for both male and female animals.

: - Sensory reflex test: Both auricle reflex test and corneal reflex test were performed evaluating sensory reflex; no specific reaction was observed in comparison with the control group.
- Motor function test: Significant decrease was observed in female 125 and 500 mg/kg b.w./day treatment groups. But these decreased values were higher than male control group since the mean value of female control group was higher than the male control group.
There was no significant result in female 250 mg/kg b.w./day group and all male rats. Because there were no dose-dependent changes, motor function was not considered to be affected by iron dichloride.
Urinalysis: There were no specific findings.
- Analysis of hematological and biochemical test of blood: statistically significant differences were found in mean cell volume (MCV), eosinophils (EOS), platelet (PLT), cholinesterase (CS), and triglycerides (TG). But these were within the biologically normal range and no dose-dependent changes were evident.
- Organ weights: Both absolute and relative weights of liver were increased in 250 and 500 mg/kg b.w./day male groups and in 500 mg/kg b.w./day female group. Also, for male rats, absolute adrenal glands weights were increased in 500 mg/kg b.w./day group, and relative adrenal glands weights were increased in 250 and in 500 mg/kg b.w./day group. Because of hemosiderin deposit in hepatocyte and hyperplasia of zona fasciculate in adrenal cortex, the increased weights of liver and adrenla glands were influnced by the test substance. In 125 mg/kg b.w./day male group, liver weight did not differ from the control group, but adrenal glands weights were decreased as compared to the control group.
For thymus, absolute weight was dicreased in female 125 and 500 mg/kg b.w./day groups, and relative weight was decreased in 500 mg/kg

b.w./day group. However, these changes were considered to be individual variations and not due to the test substance.
- Necropsy opinions:
Diaphragmatic nodules of liver were sporadically noted in the control and the treated groups. It is a congenital malformation, which is a morphological change and doesn't have physiological effects.
The following necropsy opinions were caused by the test substance; severe diffuse hemorrhagic grandular stomach and severe distension of stomach in dead animals, and diffuse black colored liver and hemorrhage with diffuse black pigmentation in schedualed necropsy of 500 mg/kg b.w./day male group. For females, a case of mass of mesenteric lumph node was observed in 500 mg/kg b.w./day group.
- Histopathology: For 500 mg/kg b.w./day groups of both sexes, hemosiderin deposit of hepatocyte and grandular, hyperplasia of zona fasciculate in adrenal cortex, hyperkeratosis of forestomach, hemosiderin deposit of grandular stomach, neutrophil infiltration of submusoca were observed. These conditions were induced by the test substance and were weaker in females. There was no specific findings in the recovery groups.
In case of dead rats, due to severe villous atrophy of forestomach, gastric function was abnormal. Therefore, it was concluded that female rats were dead by a physical irritance of test substance.

Table. Summary of the preliminary test to determine administration dosages

Dose (mg/kg)	Sex	Animal Number	Observed clinical signs	Mortality (%)	Necropsy
60	Male	PM01 PM02	NAD* NAD	0/2 (0)	NGF NGF
	Female	PF01 PF02	NAD NAD	0/2 (0)	NGF NGF
125	Male	PM03 PM04	NAD NAD	0/2 (0)	NGF NGF
	Female	PF03 PF04	NAD NAD	0/2 (0)	NGF NGF
250	Male	PM05 PM06	Salivation at day 6 NAD	0/2 (0)	NGF H, DBF
	Female	PF05 PF06	NAD NAD	0/2 (0)	NGF NGF
500	Male	PM07 PM08	Soft stool during day2 - day 6 Salivation at day 6 Soft stool during day2 - day 6 Salivation at day 6	0/2 (0)	H, DBF H, DBF
	Female	PF07	NAD	0/2 (0)	H, DBF
		PF08	NAD		NGF
1,000	Male	PM09	Dead	2/2 (100)	H, DBF
		PM10	Dead		H, DBF
	Female	PF09	Dead	1/2 (50)	H, DBF
		PF10	Soft stool during day1 - day 6 Salivation at day 6		H, DBF

* NAD: No Abnormalities Detected; NGF: No Gross Findings; H: Hemorrhage in stomach; DBP: Diffused Black Pigmentation in stomach

Table. Mortality of females (group): each animal was found dead on day 38, day 46 and day 51 of treatment, respectively.

Group:	G1	G2	G3	G4
Test article:	------	Iron dichloride		-------
Dose level (mg/kg/day):	0	125	250	500

Group	No. of animals	Week after treatment								
		1	2	3	4	5	6	7	8	Mortality (dead/total)
G1	20	0	0	0	0	0	0	0	0	0 % (0/20)
G2	15	0	0	0	0	0	0	0	0	0 % (0/15)
G3	15	0	0	0	0	0	0	0	0	0 % (0/15)
G4	20	0	0	0	0	0	0	2	1	15 % (3/20)

Table. Clinical signs (Frequency)

Group/Dose (mg/kg)	No. of animals	Signs	Frequency (%)	
			Male	Female
G1 0	20	Alopecia - forelimb	0/20 (0)	1/20 (5.0)
G2 125	15	Salivation Black stool Soft stool Diarrhea Alopecia - forelimb Alopecia - Region around ear and eye	13/15 (86.7) 15/15 (100) 1/15 (6.7) 0/15 (0) 0/15 (0) 0/15 (0)	8/15 (53.3) 15/15 (100) 0/15 (0) 1/15 (6.7) 1/15 (6.7) 1/15 (6.7)
G3 250	15	Salivation Black stool Soft stool Locomotion activity decrease	15/15 (100) 15/15 (100) 2/15 (13.3) 0/15 (0)	15/15 (100) 15/15 (100) 0/15 (0) 1/15 (6.7)
G4 500	20	Salivation Black stool Soft stool Diarrhea Locomotion activity decrease Paleness Emaciaition Soiled perineal region Death	20/20 (100) 20/20 (100) 6/20 (30.0) 1/20 (5.0) 10/20 (50.0) 1/20 (5.0) 1/20 (5.0) - 0/20 (0)	20/20 (100) 20/20 (100) 3/20 (15.0) 3/20 (15.0) 20/20 (100) 3/20 (15.0) 1/20 (5.0) 1/20 (5.0) 3/20 (15.0)

Table. Body weights of males (group)

Group:		G1	G2	G3	G4
Test article:		------	Iron dichloride		-------
Dose level (mg/kg/day):		0	125	250	500

Group	Day after treatment							
		1	7	14	21	28	35	42
G1	Mean	284.5	324.2	362.9	385.9	413.7	434.5	457.1
	SD	6.9	7.3	7.2	9.6	7.3	9.3	13.8
	N	15	15	15	15	15	15	15
G2	Mean	285.8	326.7	363.2	389.3	415.2	435.6	454.3
	SD	6.4	8.9	12.3	14.2	14.9	18.8	19.0
	N	15	15	15	15	15	15	15
G3	Mean	284.5	319.2	347.8*	368.6*	385.7*	403.6*	420.0*
	SD	6.8	10.8	15.8	20.1	19.8	21.3	22.3
	N	15	15	15	15	15	15	15
G4	Mean	285.2	300.0*	333.3*	344.6*	364.9*	376.3*	393.3*
	SD	6.5	25.1	21.3	19.3	18.9	21.7	26.1
	N	15	15	15	15	15	15	15

*: Statistical significance was observed.

Table. Body weights of females (group)

Group:		G1			G2		G3		G4	
Test article:		------			Iron dichloride				-------	
Dose level (mg/kg/day):		0			125		250		500	
Group		Premating period			Gestation period				Lactation period	
		1	7	14	0	7	14	20	0	4
G1	Mean	204.2	218.4	233.5	240.7	274.8	307.1	397.6	305.7	320.2
	SD	7.6	9.2	8.2	8.8	10.6	11.6	21.9	12.6	16.1
	N	15	15	15	11	11	11	11	11	11
G2	Mean	202.1	213.8	229.5	234.4	261.0*	293.9	383.8	291.3	297.9*
	SD	5.9	8.3	13.4	11.3	8.8	9.3	14.5	9.3	9.2
	N	15	15	15	12	12	12	12	12	12
G3	Mean	203.2	218.9	230.9	238.7	269.1	301.8	393.8	301.7	306.7
	SD	7.2	6.8	9.1	11.1	13.2	13.5	26.2	15.2	15.9
	N	15	15	15	14	14	14	14	14	14
G4	Mean	202.3	207.7*	228.8	232.2	266.7	301.0	392.1	301.2	299.0*
	SD	4.9	18.0	11.6	10.9	9.8	13.6	21.1	17.0	19.9
	N	15	15	15	10	10	10	10	10	10

*: Statistical significance was observed.

Table. Water consumption of male rats (Group) (mL/animal/day)

Group/Dose (mg/kg)	Day after treatment				
		0	6	13	40
G1	Mean	30.06	35.87	41.05	44.35
0	S.D.	4.03	6.65	10.22	16.35
	N	10	10	10	10
G2	Mean	29.51	35.36	39.78	43.42
125	S.D.	8.62	5.05	9.20	5.66
	N	8	8	8	8
G3	Mean	31.34	33.73	39.07	36.78
250	S.D.	4.56	4.74	8.96	5.00
	N	8	8	8	8
G4	Mean	31.93	45.29 *	57.04 *	38.10 *
500	S.D.	3.48	7.56	12.20	23.08
	N	10	10	10	10

N: Number of cages
*: Statistical significance was observed.

Table. Water consumption of female rats (Group) (mL/animal/day)

Group / Dose (mg/kg)		Premating period			Gestation period				Lactation period	
		0	6	13	0	6	13	20	0	3
G1	Mean	54.98	53.21	61.45	31.98	39.04	44.36	48.85	41.26	56.25
0	S.D.	9.06	13.90	9.61	4.91	7.17	9.54	9.97	8.18	22.95
	N	10	10	10	11	11	11	11	11	11
G2	Mean	53.43	52.14	60.49	30.06	35.91	41.67	48.02	44.80	65.99
125	S.D.	16.89	18.55	19.20	4.64	7.36	9.55	9.69	7.13	20.21
	N	8	8	8	12	12	12	12	12	12
G3	Mean	58.21	58.00	68.64	32.59	40.41	44.87	50.43	47.45	66.83
250	S.D.	10.44	10.59	6.92	6.35	9.47	6.56	8.73	9.27	15.07
	N	8	8	8	14	14	14	14	14	14
G4	Mean	58.16	81.04*	82.60*	36.10	47.62	47.20	65.01*	51.81*	66.33
500	S.D.	8.14	10.68	11.51	6.91	8.04	10.53	13.43	12.22	26.55
	N	10	10	10	10	10	10	10	10	10

N: Number of cages (pre-mating period), Number of animals (Gestation period & Lactation period)
*: Statistical significance was observed.

Table. Absolute organ weights of males (group mean)

Dose (mg/kg)	0	125	250	500
Brain (g)	2.11	2.01	2.02	1.95
Heart (g)	1.39	1.34	1.30	1.25
Liver (g)	11.32	11.96	13.30*	14.04*
Spleen (g)	0.90	1.03	1.03	0.89
Thymus (g)	0.49	0.49	0.47	0.36
Lt. Kidney (g)	1.41	1.44	1.42	1.36
Rt. Kidney (g)	1.44	1.45	1.46	1.39
Lt. Adrenal gland (g)	27.00	26.64	28.90	33.32*
Rt. Adrenal gland (g)	24.66	24.76	28.90	34.26*
Lt. Testes (g)	1.70	1.73	1.70	1.73
Rt. Testes (g)	1.70	1.76	1.92	1.77
Lt. Epididymis (g)	0.60	0.61	0.62	0.59
Rt. Epididymis (g)	0.60	0.62	0.60	0.58

Number of animals: 5/group
*: Statistical significance was observed.

Table. Absolute organ weight of females (group mean)

Dose (mg/kg)	0	125	250	500
Brain (g)	1.93	1.97	1.91	1.93
Heart (g)	1.03	0.97	0.96	0.98
Liver (g)	9.77	9.81	10.22	11.36*
Spleen (g)	0.86	0.73	0.72	0.74
Thymus (g)	0.34	0.23*	0.27	0.25*
Lt. Kidney (g)	0.89	0.88	0.86	0.94
Rt. Kidney (g)	0.88	0.89	0.86	0.96
Lt. Adrenal gland (g)	3.50	3.53	3.85	3.95
Rt. Adrenal gland (g)	3.47	3.42	3.46	3.78

Number of animals : 5/group
*: Statistical significance was observed.

Table. Histopathological findings

Organs	Sex	Male				Female			
	Group	G1	G2	G3	G4	G1	G2	G3	G4
	Dose(mg/kg)	0	125	250	500	0	125	250	500
	Number of examined animals	15	15	15	15	15	15	15	15
Adrenals	No. of examined	5	15	15	15	5	15	15	15
	Not Remarkable	5	15	15	6	5	15	15	11
	Remarkable	5	0	0	9	0	0	0	4
- Cortex	- Zona fasciculata, hyperplasia								
	unilateral	0	0	0	4	0	0	0	0
	bilateral	0	0	0	4	0	0	0	3
	- Zona reticulosa, hyperplasia, bilateral	0	0	0	0	0	0	0	1
- Medulla	- Necrosis, focal, unilateral	0	0	0	1	0	0	0	0

Liver	No. of examined	6	15	15	15	5	15	15	15
	Not Remarkable	5	15	15	0	5	15	13	9
	Remarkable	1	0	0	15	0	0	2	6
- Par	- Vacuolization,cytoplasmic,multifocal	1	0	0	0	0	0	0	0
enchyma	- Microgranuloma, focal	0	0	0	0	0	0	1	0
	- Deposit, hemosiderin, focal								
	minimal	0	0	0	0	0	0	0	4
	mild	0	0	0	0	0	0	0	0
	moderate	0	0	0	0	0	0	0	0
	- Deposit, hemosiderin, diffuse								
	minimal	0	0	0	2	0	0	1	1
	mild	0	0	0	8	0	0	0	1
	moderate	0	0	0	4	0	0	0	0
	severe	0	0	0	1	0	0	0	0
	- Hypertrophy, mild	0	0	0	1	0	0	0	0
Lymphnod,	No. of examined	-	-	-	-	-	-	-	1
mesenteric	Not Remarkable	-	-	-	-	-	-	-	0
	Remarkable	-	-	-	-	-	-	-	1
	- Deposit, hemosiderin, multifocal, mild	-	-	-	-	-	-	-	1
Stomach	No. of examined	5	-	-	11	5	-	-	1
	Not Remarkable	5	-	-	11	5	-	-	0
	Remarkable	0	-	-	0	0	-	-	1
	- Gastritis								
	moderate	0			1	0			0
	severe	0			2	0			0
- Grandular	- Atrophy, severe	0	-	-	0	0	-	-	1
	- Deposit, hemosiderin, diffuse								
	moderate	0	-	-	9	0	-	-	0
	severe	0	-	-	2	0	-	-	0
	- Submucosa, cellular infiltration, neutrophil, diffuse, moderate	0	-	-	3	0	-	-	0
- Fore	- Hyperkeratosis								
stomach	mild	0	-	-	7	0	-	-	0
	moderate	0	-	-	1	0	-	-	0
	severe	0	-	-	3	0	-	-	1
Testes /	No. of examined	5	-	-	5	5	-	-	5
Ovaries	Not Remarkable	5	-	-	4	5	-	-	5
	Remarkable	0	-	-	1	0	-	-	0
- Semiferous tubu	- Atrophy, tubular, unilateral	0	-	-	1	0	-	-	0

-: Not examined
Following organs did not have remarkable result; cerebellum, cerebrum, epididymides, heart, kidney, spleen, thymus, uterus.

Table. Histopathological findings (Recovery groups)

Organs	Sex	Male		Female	
	Group	G1	G4	G1	G4
	Dose(mg/kg)	0	500	0	500
	Number of examined animals	5	5	5	5
Adrenals	No. of examined	-	5	-	5
	Not Remarkable	-	5	-	5
	Remarkable	-	0	-	0
	- Congestion, diffuse	-	0	-	2
Liver	No. of examined	2	5	-	5
	Not Remarkable	2	5	-	3
	Remarkable	0	0	-	2
- Parenchyma	- Deposit, hemosiderin, focal,	0	0	-	1
	- Oval cell hyperplasia, focal	0	0	-	1
Stomach	No. of examined	-	-	-	2

	Not Remarkable	-	-	-	0
	Remarkable	-	-	-	2
- Grandular	- Atrophy, severe	-	-	-	1
- Forestomach	- Hyperkeratosis, severe	-	-	-	1

-: Not examined

Conclusions : By the particular test results such as the rate of body weight gain, water consumption, organ weights, necropsy, and histopathology, NOAEL values were determined to 125 mg/kg b.w./day for males and 250 mg/kg b.w./day for females.
Reliability : (1) Reliable without restrictions
Flag : Critical study for SIDS endpoint
Reference : (42)

Type : TDLo
Species/strain : Rat
Route of administration : Oral (drinking)
Exposure period : 30 days
Frequency of treatment : Continuously
Value : 6,604 mg/kg/30D-C
Method : Others
Year : 1991
GLP : No data
Test substance : Other TS; Iron dichloride (7758-94-3)
Results : TDLo: 6604 mg/kg/30D-C
Dose Received: 6604 mg/kg
Hematology:
- changes in serum composition (TP, bilirubin, cholesterol, etc.)
Clinical Chemistry:
- Enzyme inhibition, induction, Changes in blood or tissue levels (Phosphatase)
Histopathology:
- Liver: other change
Peroxisome Assay:
Reliability : (3) Not reliable
Reference : (43)

Type : TCLo
Species/strain : Rat
Route of administration : Inhalation
Exposure period : 65 days
Frequency of treatment : Continuously
Value : 200 $\mu g/m^3$/24H/65D-C
Method : Others
Year : 1985
GLP : No data
Test substance : Other TS; Iron dichloride (7758-94-3)
Results : TCLo: 200 $\mu g/m^3$/24H/65D-C
Dose Received: 200 $\mu g/m^3$
Hematology: - Changes in serum composition (TP, bilirubin, cholesterol)
Clinical Chemistry: - Enzyme inhibition, induction, or change in blood or tissue levels (true cholinesterase)
Brain and Coverings – other degenerative changes
Conclusions :
Reliability : (3) Not reliable
Reference : (44)

5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

Type	:	Bacterial reverse mutation assay
Species/Strain	:	*Salmonella tryphimurium*(strains TA 98, TA 100, TA 1535 and TA 1537) and *Escherchia coil* (strain WP2 *uvr*A)
Method	:	OECD TG 471 "Bacterial Reverse Mutation Test"
System of testing	:	Bacterial
Year	:	2004
GLP	:	Yes
Metabolic activation	:	- Species and cell type: Rat (Sprague Dawley strain), male, liver - Quantity: 5 % S9 mix induced with Aroclor 1254
Concentrations tested	:	33.3, 100, 300, 1,000, 3,000 and 5,000 μg/plate
Statistical Methods	:	Dunnett's test
Test substance	:	Other TS: Iron dichloride, purity = 98 %, Sigma-Aldrich corporation, Lot No. - 23828CB
Test conditions	:	Pre-incuvation method was performed with triplicate plates both in the presence and absence of metabolic activation system. Preliminary tests were carried out using dose levels with 5-fold intervals of 1.6, 8, 40, 200, 1,000 and 5,000 μg/plate to determine the dose range. Positive and negative control groups and treatment: Negative control - vehicle control (Dimethylsulfoxide), Positive control – 2-Nitrofluorene, Sodium azide, 9-Aminoacridine, 4-Nitroquinoline and 2-Aminoanthracene Solvent: Dimethylsulfoxide Criteria for evaluating results: Significant increase in the number of revertant colonies for at least one strain at one or more concentrations.
Results	:	
Cytotoxic conc.		With metabolic activation: not observed Without metabolic activation: at 5,000 μg/plate (TA 98 and TA 100)
Genotoxic effects		With metabolic activation: negative Without metabolic activation: negative
Statistical results		Dunnett's test was used to compare the test groups with the control groups. High statistical significance was only observed in positive control groups. Precipitation concentration: at and above 1,000 μg/plate

Table. Results of bacterial reverse mutation assay with iron dichloride

Tester strain	Chemical treated	Dose (μg/plate)	Colonies/plate (mean ± SD)	
			Without S9 mix	With S9 mix
TA 98	Test item	0	30 ± 4	29 ± 8
		33.3	30 ± 6	24 ± 1
		100	29 ± 4	32 ± 10
		300	28 ± 4	26 ± 5
		1,000	18 ± 6 *	27 ± 7 *
		3,000	19 ± 4 *	26 ± 8 *
		5,000	6 ± 1 *	30 ± 11 *
TA 100	Test item	0	88 ± 13	97 ± 17
		33.3	67 ± 6	91 ± 8
		100	78 ± 3	75 ± 3
		300	72 ± 7	92 ± 14
		1,000	77 ± 8 *	82 ± 3 *
		3,000	61 ± 15 *	117 ± 12 *
		5,000	25 ± 2 *	111 ± 33 *
TA 1535	Test item	0	18 ± 3	12 ± 3
		33.3	10 ± 3	10 ± 2
		100	16 ± 4	15 ± 1
		300	12 ± 1	11 ± 2
		1,000	13 ± 0 *	10 ± 1 *
		3,000	9 ± 3 *	10 ± 4 *
		5,000	7 ± 2 *	12 ± 4 *
TA 1537	Test item	0	12 ± 3	11 ± 3
		33.3	21 ± 1	9 ± 4
		100	15 ± 8	7 ± 1
		300	9 ± 4	8 ± 4
		1,000	7 ± 4 *	10 ± 4 *
		3,000	3 ± 2 *	7 ± 3 *
		5,000	3 ± 2 *	6 ± 3 *
E. coli WP2 *uvr*A	Test item	0	14 ± 1	14 ± 4
		33.3	12 ± 5	13 ± 2
		100	14 ± 3	14 ± 1
		300	11 ± 5	9 ± 2
		1,000	12 ± 0 *	13 ± 4 *
		3,000	12 ± 2 *	8 ± 1 *
		5,000	10 ± 4 *	12 ± 5 *
Positive control				
TA 98	2-NF	1.0	245 ± 24 SS	
	2-AA	2.0		631 ± 26 SS
TA 100	SA	0.5	404 ± 18 SS	
	2-AA	2.0		905 ± 31 SS
TA 1535	SA	0.5	338 ± 16 SS	
	2-AA	5.0		216 ± 16 SS
TA 1537	9-AA	50	276 ± 34 SS	
	2-AA	5.0		263 ± 24 SS
WP2 *uvr*A	4-NQ	2.0	598 ± 28 SS	
	2-AA	10		283 ± 28 SS

*; precipitation
SS; Statistical significance was observed ($p \leq 0.01$)

2-NF; 2-Nitrofluorene, 2-AA; 2-Aminoanthracene, SA; Sodium azide, 9-AA; 9-Aminoacridine, 4-NQ; 4-Nitroquinoline

Conclusions : Iron dichloride did not cause the reverse mutations in the *Salmonella tryphimurium*(strains TA 98, TA 100, TA 1535 and TA 1537) and *Escherichia coli* (strain WP2 *uvr*A).
Reliability : (1) Reliable without restrictions
Flag : Critical study for SIDS endpoint
Reference : (45)

Type : Rec assay
Species : *Bacillus subtilis*
Strain : H17 (Rec^+, *arg*$^-$ and *trp* -), M45 (Rec^-, *arg* $^-$ and *trp* $^-$)
Method : Other
System of testing : Bacteria
Year : 1975
GLP : No
Metabolic activation : Not used
Concentration tested : 0.05 M
Statistical Methods : Not used
Test substance : Other TS: Iron dichloride (CAS No. 7758-94-3)
Remarks: Source: Nakarai Chemical Ltd.,Kyoto, Japan, Grade and purity not stated
Test conditions : Number of tests: at least triplicates
Positive and negative control: not stated
Solvent: distilled water. If insoluble in water, added solutions of NaOH(1 N) or HCl(1 N).
Criteria for evaluating results: The inhibition zone was indicated by distance(mm) between the edge of the paper disc conitaining test substance and that of bacterial streaks. The difference between Rec^+ and Rec^- cells may indicate the mutagenicity basede on DNA damaging capacity.
Remarks : *Methods:* Strains of *Bacillus* cultures were streaked on NB agar from a central point to different directions using 0.1 ml pipettes. An 0.05 ml aliquot of each metal solution (0.05 M) was dropped on to a filter paper disc (diameter 10 mm) which had been placed at the starting point of the streaks of Rec^+ and Rec^- bacteria. All the plates were then incubated at 37 °C for 24 hr.
Result :

Table. A difference of rec-effect by iron dichloride

Test substance	Inhibition zone in strains of *Bacillus subtilis* (mm)		Rec-effect of difference in inhibition
	H17(Rec^+)	M45(Rec^-)	
Iron dichloride	1	1	-

Conclusions : Iron dichloride did not show mutagenic activity.
Reliability : (2) Reliable with restrictions
References : (46)

Type : Rec assay
Species : *Bacillus subtilis*
Strain : H17 (Rec^+, *arg*$^-$ and *try* -), M45 (Rec^-, *arg* $^-$ and *try* $^-$)
Method : Other
System of testing : Bacteria
Year : 1980
GLP : No
Metabolic activation : Not used
Concentrations tested : 0.005 - 0.5 M

		Not specified for metals with negative results.
Statistical Methods	:	Not used
Test substance	:	Other TS: Iron dichloride (CAS No. 7758-94-3) Remarks: Source: Maruichi Chemical Ltd.,Misima, Japan, Used the highest purity avilable.
Test conditions	:	Number of tests: not specified Positive and negative control: not stated Solvent: distilled water Criteria for evaluating results: DNA-damaging capacity was estimated by comparing the growth inhibitions for Rec^+ and Rec^- strains.
Remarks	:	*Methods:* Two strains of *Bacillus subtilis* were streaked radially onto B-2 agar. A 0.05 ml of each metal solution was dropped onto a filter paper disk (diameter 10 mm), and the disk was placed on the starting point of the streak. The plates were kept at 4 °C for 24 h and then incubated 37 °C overnight. The cold incubation prior to 37 °C incubation increases the assay sensitivity about 20 – 50 times for many drugs.
Result	:	Iron dichloride was negative in the rec assay.
Conclusions	:	The mutagenic activity did not occur.
Reliability	:	(2) Reliable with restrictions
References	:	(47)
General remarks	:	Rec assay to assess the potential genetic toxicity of chemicals is based on the theory that if the chemical is damaging cellular DNA, growth of recombination-repair-deficient (Rec-) bacteria is more inhibited than that of wild type bacteria (Rec+). Many studies performed both Rec assay and other in vitro genetic toxicity tests suggested that Rec assay gave more sensitive results than bacterial reversion assays. In this study, 127 metal compound were tested. All the rec-assay-positive metal compounds were tested with the reverse mutation induction assays with E. coli and Salmonella strains. Less than 30 % of the rec-assay-positive compounds showed positive effects in the reversion assays.

Type	:	Baterial reverse mutation assay
Species	:	*Salmonella tryphimurium*
Strain	:	TA 1537, TA 2637, TA 98, TA 100 and TA 102
Method	:	Other
System of testing	:	Bacteria
Year	:	1987
GLP	:	No
Metabolic activation	:	Not used
Concentrations tested	:	1 – 10,000 μmoles/plate
Statistical Methods	:	Not used
Test substance	:	Other TS: Iron dichloride Remark: Used highest grade available
Test condition	:	Solvent: water
Remarks	:	*Methods* Mutation assay: The test cells were washed twice by centrifugation to remove metal compounds. The cells were subsequently resuspended in Tris-HCl buffer. An aliquot of the suspension was then added to 2 ml of molten soft agar and poured before plating onto a Tris minimal agar plate. The same mutation assays were done in the presence of 9-aminoacridine(9AA).
Results	:	Iron dichloride was found to be non-mutagenic in all five strains, but showed mutagenic activity when tested together with 9AA in two strains, TA 1537 and TA 2637.
Conclusions	:	Iron dichloride alone was non-mutagenic in strain TA 1537, TA 2637, TA 98, TA 100 and TA 102.
Reliability	:	(3) Not reliable
Reference	:	(48)
General remarks		All 21 different metal chlorides tested were non-mutagenic by themselves

in all 5 strains. When tested with 9AA, seven compounds increased the mutagenic activity of 9AA in TA 1537 and TA2637 but not in TA 98, TA 100 and TA 102.

B. NON-BACTERIAL IN VITRO TEST

Type	:	Comet assay
Species/Strain	:	Human lymphocytes
Method	:	Other
Year	:	2000
GLP	:	No
Metabolic activation	:	Not used
Concentrations tested	:	0, 5, 10, 30, 50, 100, and 300 μM
Statistical Methods	:	See below test condition
Test substance	:	Other TS: Iron dichloride(CAS No.: 7758-94-3) Remarks: Source, grade, and purity not stated
Test conditions	:	Number of replicated: repeated on several occasions but only representative experiments were shown. Slides were prepared in duplicates. Positive control: treated with H2O2 Negative control: not treated cells Solvent: not stated Criteria for evaluating results Statistics: The 75th centile tail moment value for 25, 50, or 100 randomly selected cells per slide was used to present comet assay data. Dose-squared regression analysis were used to examine the dose relationships of 50 cells per dose levels. ANOVA was used to compare responses from the different combinations of chemicals. Friedman's test was used in the same way as the ANOVA, but for the values obtained for the 75th centile of comet tail moments.
Remarks	:	Methods - Cell preparation (Lymphocytes): Blood samples were obtained from a male and female donor. Lymphocytes were separated by centrifugation on a Ficoll density gradient and were suspended in 75 μl low melting point agarose (LMA) for embedding on slides. Cells were checked for viability by trypan blue exclusion. - Cell treatment: Single cell suspension of lymphocytes was treated for 30 min at 37 °C in serum free RPMI 1640 (Rothwell Park Memorial Institute). - Slide preparation: Around 10,000 treated or control cells were mixed with 75 μl of 0.5 % LMA to form a cell suspension and spread on agrose layer on a microscope slide. The cells were covered with top agosrose layer and were immersed in lysis solution. - Electrophoresis: The slides were placed in a horizontal gel electrophoresis tank were left in the EDTA/NaOH solution for 40 min to allow the unwinding of the DNA. Electrophoresis was conducted at 4 °C for 20 min, using 26 V under dimmed light to prevent the additional DNA damage. The slides were neutralized in a Tris buffer, pH 7.5 and stained with Ethidium Bromide solution. - Slide scoring: Slides were examined under the fluorescence microscope. - Analysis by computer: A computerized image analysis system was used to measure comet parameters.
Results	:	

Statistical results

Table. Responses in lymphocytes

Chemical	Experiment donor			
	1 Female	2 Female	3 Female	1 male

H_2O_2	High significant regression	High significant regression	High significant regression	High significant regression
$FeCl_2$	No significant regression	High significant regression	No significant regression	High significant regression
H_2O_2 + $FeCl_2$ compared with H_2O_2	No significant anova	High significant anova	No significant anova	-

The lymphocytes from the male and female donors showed DNA damage increases after treatment with H_2O_2 ($p < 0.05$). Iron dichloride alone in human lympocytes produced little DNA damage.

Conclusions : Iron dichloride caused weak DNA damage in human lymphocytes, but it did not produce a dose-related response.

Reliability : (3) Not reliable,

Reference : (49)

Remarks : In this simulated iron overload studies, human cells were treated with an agent that can cause oxygen radical damage (H_2O_2) in combination with iron compounds. This latter agent was either a ferrous of ferric salt or haemosiderins isolated from a thalassaemic patient.

Type : Viral enhancement assay

Species/Strain : Primary Syrian hamster embryo cells(HEC) / Simian adenovirus SA7

Method : Other

System of testing : Virus

Year : 1979

GLP : No

Metabolic activation : Not used

Concentrations tested : 0, 0.6, 1.2, 2.5 and 5.0 mM

Statistical Methods : Lorenz table which is based upon the Poisson distribution

Test substance : Other TS: Iron dichloride (CAS No. 7758-94-3)
Remarks: Source, grade and purity not stated
Stock solutions were made in acetone: water (1:1)

Remarks : *Methods:*
- Cell culture: Primary HEC was prepared by trypsinization of eviscerated and decapitated embryos after 13 to 14 days of gestation.
- Virus: Simian adenovirus SA7 was inoculated in Vero or BSC-1 cell cultures. Cytopathic effects were usually complete by 72 hrs, after which the infected cells were harvested and freeze-thawed 4 times. The virus was separated by low-speed centrifugation and stored in 1 or 2 ml aliquots at -65 °C.
- Transformation assays: SA7 was inoculated to HEC cells ($3 – 4 \times 10^7$ PFU/culture) and adsorbed for 3 hrs; the virus-infected cells were replated at 2×10^5 cells/60mm dish; After incubation for 3 days, the medium was changed to MDM with 0.1 mM $CaCl_2$, 10 % FBS, and $NaHCO_3$ (0.22 g / 100 ml). After 6 days, plates were overlaid with 3 ml of the above medium containing Bacto-agar (0.3 g / 100 ml). At intervals of 4, 5, and 6 days, 3 ml of additional agar medium were added. After 25 to 30 days from the beginning of the experiment, the final number of SA7 foci was counted.
- Survival assays: Five dishes with 666cells/dish were incubated for five to 6 days and 3 ml of medium with double the amount of $NaHCO_3$ were added to each plate. After 8 to 9 days of total incubation period the cells were fixed in 10 % buffered formalin and stained with 0.02 % crystal violet.
- Chemical treatment: In each experiment, 2 plates of HEC were treated with metal salts for 18 hrs prior to inoculation, or 5 to 10 plates were treated for 5 hrs after virus inoculation. After pretreatment, HEC were rinsed with complete medium and inoculated with SA7. Transformation and clonal assays were performed with each treatment groups. When

treated after virus inoculation, the metal salts remained in the medium for 48 hrs.
- Determination of enhancement: Surviving fraction was determined by dividing the number of colonies from treated cells by those of colonies from control cells. Enhancement ratio was calculated by dividing the TF of treated cells (TF = SA7 foci x reciprocal of the surviving fraction) by that obtained from control cells.
- Criteria for evaluating results: The increased TF was considered statistically significant at the 5 or 1 % confidence level if the enhancement ratio exceeded the appropriate values obtained from the Lorenz table.

Result :

Table. Enhancement ratio of iron dichloride

Chemical	Conc. (mM)	Surviving fraction	SA7 foci	Enhancement ratio
	5.0	0.56	61	3.7 b
	2.5	0.62	51	2.7 b
FeCl2	1.2	0.63	36	1.9 a
	0.6	0.67	34	1.7
	0	1.00	30	1.0

[a] Significant at the 5 % level
[b] Significant at the 1 % level

Statistical results: Statistically significance at the 5 or 1 % confidence level was observed at and above 1.2 mM.

Conclusions : Iron dichloride enhanced virally induced cell transformation at and above 1.2 mM.
Reliability : (3) Not reliable
References : (50)
General remarks : Cell transformation is highly relevant to carcinogenesis in vivo

Type : In vitro assay
Species : pz189 (5504 bp) DNA
Method : Other
System of testing : DNA
Year : 1992
GLP : No
Metabolic activation : Not used
Concentrations tested : Low concentration of iron dichloride was used. Not specified.
Statistical Methods : Not used
Test substance : Other TS: Iron dichloride (CAS No. 7758-94-3)
Remarks: Source: Mallinckrodt, Paris, KN, grade and purity not stated.
Test conditions : Number of replicated: one
Solvent: water
Criteria for evaluating results: based upon the differential mobility of different forms of plasmid DNA; supercoiled, linear, and circular form
Remarks : Methods: Supercoiled plamid DNA (0.5 ug) in 10 mM phosphate buffered saline, pH 7.4, was incubated for 30 min at 24 °C with iron dichloride. Plasmid DNA and phosphate buffer were pretreated with Chelex resin, sodium form(Sigma; 20 %, v/v), to minimize metal contamination. Iron dichloride solutions were made with water through which nitrogen was bubbled for 1 hr, and used immediately. Samples were analyzed by electrophoresis in 0.8 % agarose gel and the gel was stained with ethidium bromide, photographed, and scanned with a densitometer. Peak area was integrated and DNA strand breaks were evaluated.
Results : Fe(II) induced decrease in supercoiled forms and increase in linear and circular forms by DNA breakage.
Conclusions : Fe(II) alone induced DNA strand breakage.
Reliability : (3) Not reliable
Reference : (51)
General remarks : The assay, based on the relaxation and linearization of supercoiled DNA

is a simple yet sensitive and quantitative test for DNA damage.

Type	:	In vitro assay
Species/Strain	:	HeLa S3 cells
Method	:	Other
Year	:	1996
GLP	:	No
Metabolic activation	:	Not used
Concentrations tested	:	0.1, 0.3, and 1 mM
Statistical Methods	:	Not used
Test substance	:	Chloride salts of metal Remarks: Source: Sigma, grade and purity not stated
Remarks	:	Molar stock solution: dissolved in deionized water. Incision assay: 200 ng each fo UV-treated pBS plasmids and untreated pHM14 control plasmids were incubated for 2h at 30 °C with 200 ug divalent cation-free HeLa protein extracts in the presence of 7 mM MgCl2 and other divalent (0.1, 0.3, and 1 mM) metal ions. The plasmids were labeled in the presence of [α-32P]dATP and Klenow fragment and analysed by agarose gel electrophoresis after linierlized by HindIII. The repair incision activity was estimated by the ratio of radiolabled nucleotide incorporations in UV-damaged versus undamaged plasmids. Criteria for evaluating results: radio-label decrease in the UV-damaged plasmid with increasing metal concentrations visualized by autoradiography.
Results	:	When the concentration of Fe(II) was at 0.3 mM or above, the ratio of radioactive nucleotide incorporations of UV-damaged plasmids to undamaged plasmids were decreased from 5 to 1.
Conclusions	:	Fe(II) inhibited the nuclotide excision repair activity of HeLa cell extracts.
Reliability	:	(3) Not reliable
References	:	(52)
General remarks	:	Using In vitro assay, the potential interference of several metal (II) ions was assessed on the damage recognition and the strand incision/displacement steps of nucleotide excision repair (NER). The results were as follows. (1) Fe(II) inhibited specifically the incision repair activity. (2) There was a good correlation between an inhibiting effect on the incision activity and a reduced protein binding activity to a damaged DNA probe.

Type	:	DNA damage in isolated rat liver nuclei
Species/Strain	:	Rats
Method	:	Other
System of testing	:	Isolated rat liver nuclei
Year	:	1982
GLP	:	No
Metabolic activation	:	Not used
Concentrations tested	:	0.75 and 1.5 mM
Statistical Methods	:	Not stated
Test substance	:	Other TS: Iron dichloride (CAS No. 7758-94-3) Remarks: Source: J. T. Baker Chemical Corp., grade and purity not stated
Remarks	:	Method: - Isolation of nuclei: isolated from the livers of 250 – 300 g male rats - [3H]dTTP incorporation into DNA: Experiments were performed with paired incubations, with and without iron. Incorporation was measured as the difference between radioactivity (d.p.m.) incorporated/μg of DNA in the iron-containing incubations minus radioactivity in the paired control. The [3H]dTTP-incorporated DNA were purified by cesium salt gradients or by acid-precipitation/alkaline-hydrolysis and analyzed. - Determination of single-strand DNA fragmentation: by alkaline elution.

Lipoperoxidation was measured by the proportionate decline in phospholipid polyunsaturated fatty acid after incubation with iron.
Analysis procedures: Phospholipid fatty acids were analysed by gas chromatograph. Iron was mesured with atomic-absorption spectrophotometer. All assays were proformed in triple-distilled and deionized water whose endogenous iron content was assessed before use. DNA was assayed by the Burton (1956) procedure. Scintillation counting was done with a Beckman LS-250 scintillation spectrometer, and gamma radioactivity of 59Fe (Amersham-Searle, Oakbrook, IL, U.S.A.) was determined with Beckman gamma counter, model 310.

Results : Iron induced fragmentation of single strand DNA: data for iron dichloride was not shown. Dose-dependent increase at 0.75 mM and 1.5 mM was detected by iron trichloride.
[3H]dTTP incorporation into DNA: Iron dichloride showed dose dependent increase at 0.75 mM to 1.5 mM; 2-fold higher than iron trichloride.

Table. Alteration in the proportions of phospholipid fatty acids as unsaturated index (the sum of each fatty acid's mole fraction multiplied by number of its unsatruated double bonds)

	Unsaturated index
Without iron	205.9
0.75 mM FeCl2	187.4
1.5 mM FeCl2	142.2
0.75 mM FeCl3	204.3
1.5 mM FeCl3	197.7

Table. Effect of thiol reagents and peroxidation inhibitors on iron dichloride (0.75 mM)-stimulated [^{3}H]dTTP incorporation into DNA

Inhibitor and its conc.	Incorporation (% of control)
α-Tocopherol (2 mM)	18 $\pm$ 5
Hydroxybutyltoluene (2 mM)	41 $\pm$ 6
Mannitol (10 mM)	28 $\pm$ 10
EDTA (2.4 mM)	10 $\pm$ 6
Catalase (20 units/0.4 ml)	43 $\pm$ 12
Superoxide dismutase (270 units/0.4 ml)	58 $\pm$ 18
Dithiothreitol (10 mM)	7 $\pm$ 0.2
Mercaptoethanol (70 mM)	16 $\pm$ 1.4
N-Ethylmaleimide (60 mM)	100 $\pm$ 1.9
5,5'-Dithiobis-(2-nitrobenzoic acid) (10 mM)	201 $\pm$ 8.3

59Fe binding to DNA of isolated nuclei:
- Iron dichloride: no information
- Iron trichloride: about 0.12 % of the applied 59Fe radioactivity was recovered from the DNA band of the gradients
- Without iron trichloride: no 59Fe found with the DNA band

Conclusions : Iron dichloride was about twice as active as iron trichloride. Lipid peroxidation took place in nuclei incubated with iron dichloride, but not with iron trichloride. Generation of reactive forms of oxygen was required for iron-mediated DNA damage, but evidence for direct interaction of reactive oxygen with DNA was not found.

Reliability : (3) Not reliable

Reference : (53)

5.6 GENETIC TOXICITY IN VIVO

Type : Mammalian erythrocyte micronucleus test

Species/Strains : Mouse/ICR (SPF)

Sex	:	Male
Method	:	OECD TG 474 “Genetic Toxicity: Micronucleus Test”
Year	:	2004
GLP	:	Yes
Route of administration	:	Intraperitoneal injection
Doses	:	0, 12.5, 25 and 50 mg/kg b.w.
Exposure period	:	24 hours
Statistical methods	:	Chi-square test(using a 2×2 contingency table)
Test substance	:	Other TS: Iron dichloride, purity = 98 %, Sigma-Aldrich Corporation, LOT No. – 23828CB
Test conditions	:	- Age at study initiation: 7 weeks - No. of animals per dose: 6 - Vehicle: Corn oil (CAS No.: 8001-30-7) - Duration of test: 3 days - Frequency of treatment: once a day for two days (only positive control group was dosed once) - Sampling times and number of samples: 24 hours after administration - Control groups and treatment: Negative control (corn oil), Positive control (2.0 mg/kg b.w. of Mitomycin C) - Clinical observations performed: Yes - Organs examined at necropsy: not examined - Criteria for evaluating results: at least 2,000 polychromatic erythrocytes per animals were scored for the incidence of micronuclei. - Criteria for selection of maximum tolerated dose (M.T.D): preliminary tests were conducted to determine the maximun tolerated dose. Two males of ICR mice were dosed at 2,000 mg/kg b.w./day and three males each were dosed at 20, 50, 100, 200, 500 and 1,000 mg/kg b.w./day dose levels once a day for two consecutive days.
Results	:	Preliminary tests: All animals of 200, 500, 1,000 and 2,000 mg/kg b.w./day dose levels and one animal of 100 mg/kg b.w./day were dead after the first dosing. Two animals of 100 mg/kg/day group and all of 50 and 20 mg/kg/day groups showed piloerection after the first dosing. After the second dosing, piloerection and hypoactivity were observed in all animals of 100 mg/kg b.w./day and piloerection was observed in all animals of 50 and 20 mg/kg b.w./day groups. One animal of 100 mg/kg b.w./day group was dead 2 days after the second dosing. Main tests: After first dosing, one animal of 50 mg/kg b.w./day dose level was dead and piloerection was observed in all animals of 25 and 50 mg/kg b.w./day groups. After second dosing, piloerection and hypoactivity were observed in three animals of 50 mg/kg b.w./day group. Any clinical sign was not observed in 12.5 mg/kg b.w./day group.

Table. Summary of PCE/(PCE+NCE) ratio and MNPCE frequency

Treatment group	PCE/(PCE+NCE) (mean)	Group mean frequency of MNPCE per 2,000 PCE (mean±S.D.)
Vehicle (10 ml/kg)	0.58	3.8 ± 2.3
Iron dichloride (12.5 mg/kg)	0.61	3.8 ± 1.6
Iron dichloride (25 mg/kg)	0.56	3.7 ± 1.5
Iron dichloride (50 mg/kg)	0.51	2.0 ± 1.0
Mitomycin C (2 mg/kg)	0.54	185.7 ± 16.3^{SS}

^{SS}: Statistical significance was observed ($p \leq 0.05$)

Genotoxic effects	:	Negative
Conclusions	:	Iron dichloride did not induce micronuclei in the mice bone marrow cells under the test conditions.
Reliability	:	(1) Reliable without restrictions

Flag	:	Critical study for SIDS endpoint
Reference	:	(54)
Type	:	Drosophila wing spot test
Species	:	Drosophila melanogaster
Strain	:	Mutiple wing hair strain (mwh jv; spapol) and flare-3 strain (flr3 /TM3, Ser)
Method	:	Other
GLP	:	No
Year	:	1994
Route of administration	:	Oral
Concentrations tested	:	20, 40, 60 and 80 mM
Statistical Methods	:	See test conditions for detail
Test substance	:	Other TS: Iron dichloride (CAS No.: 7758-94-3) Remarks: Source: Wako Pure Chemical Industries, Ltd., Osaka, The highest grade available
Remarks	:	Methods: - Drosophila stocks: The markers mwh and flr3 are recessive wing-hair mutations located on the third chromosome at 0.0 and 38.8, respectively. Mwh homozygotes produce three or more hairs per cell instead of a single smooth hair and flr3 homozygote cells produce misshapen, flare-like hairs. TM3 is a third chromosome marker with a notched wing phenotype (Ser). - Wing spot tests: Virgin mwh jv; spapol females and flr3 /TM3, Ser males were mated, and their F1 progeny were sampled at third instar larvae stage. The larvae were allowed to develop to adulthood. Spots comprising only mwh of flr hairs (single spots) and those with neighboring mwh and flr spots (twin spots) were scored as mutant clones. The spots were reclassified into two classes according to the number of mutant hairs per spot, namely, small spot with one or two mutant hairs and large spots with three or more mutant hairs. All the experiments were carried out at 25 ± 1 °C. - Test designs Number of replicated: no details Positive control: CoCl2 Negative control: no treatment Solvent: distilled water - Statistical analysis: The hypotheses were (1) the frequency of spots in the treated series was not higher than that in the control series and (2) the frequency of spots in the treated series was no less than m times the control series. The adopted m value was 2 for small spots and 5 for large spots. Both hypothese were tested at the 5 % significance level by the $\chi 2$ test with Yate's correction. - Classification for conclusions: positive (the first hypothesis rejected / the second accepted), weak positive (both hypotheses rejected), negative (the first accepted / the second rejected) and inconclusive (both accepted) - Toxicity tests: LD50 was determined from dose-survival curves. The survival, S, of treated larvae was determined at each dosage; S = St/S0 (St : the surviving fraction in the treated vial, S0: the surviving fraction in the control vial) - Criteria for evaluating results: The dose-response data and the statistical results.
Results	:	LD50 was 64 mM.

Table. Genotoxicity of iron dichloride was negative (-) through all concentrations used.

Dose (mM)	Number of wings	Small spots[a]			Large spots[b]		
		Number of spots	F[c]	D [d] (m=2)	Number of spots	F[c]	D [d] (m=5)
0	972	352	0.362		35(7) [e]	0.036	
20	116	42	0.362	-	4(1)	0.034	-
40	119	37	0.311	-	3(1)	0.025	-

60	108	41	0.380	-	3(1)	0.028	-
80	44	21	0.477	-	2(1)	0.045	-

m; multiplication factor
[a] Spots with one or two mutant hairs.
[b] Spots with three or more mutant hairs.
[c] Number of spots per wing.
[d] Statistical diagnosis
[e] Number of twin spots.

Conclusions : Iron dichloride was not genotoxic in this assay.
Reliability : (2) Reliable with restrictions
Reference : (55)
General remarks : The Drosophila wing spot test is fast and sensitive for detecting mutation and recombination in vivo. The wing spot test assesses mitotic recombination and mutational events of various kinds. Single spots are due to different genotoxic mechanisms: point mutation, deletion, chromosome breakage, and mitotic recombination; while twin spots are produced only by mitotic recombination.
In this study, genotixic activity of iron dichloride was tested with other 7 metal salts including CoCl2 which was registered as mutagen or genotoxin. CoCl2 induced spots with three or more mutant hair(large spot), CoCl2, MnCl2, MoCl3, NiCl3 and ZnCl2 were clearly effective in inducing spots with one or two mutant hairs (small spots), and CrCl3, FeCl2 and FeCl3 were non-genotoxic under the conditions used.

5.7 CARCINOGENICITY

5.8 TOXICITY TO REPRODUCTION

Species/strains : Rat (Sprague-Dawley)
Sex : Male/Female
Method : OECD TG 422 “Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test”
Year : 2004
GLP : Yes
Route of administration : Oral (Gavage)
Dose levels : 0, 125, 250 and 500 mg/kg b.w./day
Exposure period : 42 days for male animals and 42 to 54 days for female animals
Frequency of treatments : Daily
Control groups : Yes (Concurrent no treatment)
Premating exposure period : 2 weeks
Statistical methods : Same as for “repeated dose toxicity (OECD TG 422)“.
Test substance : Other TS: Iron dichloride purity = 98 %, Sigma-Aldrich Corporation, LOT No. – 14330TA

Test conditions : **Test organism**
- Sex: male/female
- Age of animals: 8 weeks old for males and females
- Weights during the study: 269.23 – 302.18 g for males and 191.34 – 221.60 g for females
- Number of test animals: 70 animals for each sex

Observation of F0
- *Number of implantation and corpus luteum*: while necropsied, the number of corpus leteum and implantation were counted; the former was measured in the ovary and the latter was measured in the uterus.
- *Mating*: Mating period was 14 days. The day after the copulating, mating was verified by sperm in a vaginal rinse. Mating rate, fertility rate for both sexes, and parturition rate were estimated.
- *Pregnancy and delivery*: The period of pregnancy was calculated from mating date (day 0). In addition, delivery period was estimated to identify the delivery (day 0).

NOAELs : 500 mg/kg b.w./day for both sexes

Results : **Results for F0**
- *Number of implantation and corpus luteum*: Pre-implantation loss rates (%) were 14.4, 9.4, 14.3, and 9.8 at the 0, 125, 250, 500 mg/kg b.w./day treatment groups, respectively. Post-implantation loss rates (%) were 6.0, 6.0, 3.1, and 7.0.
- *Estimation of mating data*: Mating rates (%) for the control group, 125, 250, 500 mg/kg b.w./day treatment groups were 93.3, 86.7, 100, and 100, respectively. Fertility rates (%) were 73.3, 80.0, 93.3, and 73.3 for male rats, and 78.6, 92.3 , 93.3, and 73.3 for female rats. Parturition rates (%) were identical with the fertility rates for female animals.
- *Pregnancy*: The period of pregnancy was 22.0, 22.1, 22.2, and 22.1 days for the control group, 125, 250, 500 mg/kg b.w./day treatment groups, respectively.

Table. Mating, fertility, gestation, and postpartum data

DOSE (mg/kg)	0	125	250	500
Pairs started (N)	14	13	15	15
Mating rate (%)	93.3	86.7	100	100
Male fertility rate (%)	73.3	80.0	93.3	73.3
Female fertility rate (%)	78.6	92.3	93.3	73.3
Parturition rate (%)	78.6	92.3	93.3	71.4*
Pre-implantation loss rate (%)	14.4	9.4	14.3	9.8
Post-implantation loss rate (%)	6.0	6.0	3.1	7.0
No. of corpora lutea	17.6	16.8	17.2	17.4
No. of implantations	15.1	15.3	14.8	15.7
No. of neonate	14.2	14.3	14.3	14.6
Mean gestation period (day)	23.6	22.1	22.3	22.1

*: Animal death before parturition was excluded in calculation of 'Parturition rate' and 'Mean gestation period'

Table. Historic control data of mating, fertility and gestation

- Mating method & method: Monogamy & 2 weeks

- Species/ train: Rat/ Sprague dawely

	No. of Male	No. of female	No. of coitus confirmed female	No. of pregnant female	Mating rate (%)	Male fertility rate (%)	Female fertility rate (%)
	15	15	14	11	93.3	73.3	78.6
	25	25	24	24	96.0	96.0	100.0
	25	25	25	25	100.0	100.0	100.0
	25	25	24	20	96.0	80.0	83.3

	16 16 21	16 16 21	16 16 21	15 16 18	100.0 100.0 100.0	93.8 100.0 85.7	93.8 100.0 85.7
Total	143	143	140	129 Mean±S.D.	97.9 97.9±2.8	90.2 89.8±10.4	92.1 91.6± 9.0

Mating rate (%) = (No. of female confiremd about coitus/ No. of male using mating) × 100
Male fertility rate (%) = (No. of pregnant female/ No. of male using mating) × 100
Female fertility rate (%) = (No. of pregnant female/ No. of female confirmed about coitus) × 100

Conclusions	:	There was no significant difference in mating data, pre and post implantation loss rates between the control group and the treatment groups. Therefore, NOAEL of reproducitve function was 500 mg/kg b.w./day for both sexes.
Reliability	:	(1) Reliable without restrictions
Flag	:	Critical study for SIDS endpoint
Reference	:	(42)

Type	:	In vitro
Species/strains	:	Sprague-Dawley Rats
Sex	:	Male
Method	:	Other, isolated rat adrenal and leydig cells
Method remarks	:	- Preparation of metal solutions The metal stock solution was prepared in sterile distilled water and sterilized by filtration. The working solution (10^{-6}, 10^{-5} and 10^{-4} M) is prepared by dilution from the stock solution in Krebs-Ringer bicarbonate buffer containing 1 mg/ml of glucose before use. - Preparation of isolated rat's Leydig cells and treatments Decapsulated testes was isolated from rats (120 – 160 g) and cells were dissociated with 1 mg/ml collagenase for 40 min. Cells were collected and then washed through repeated centrifugations (800 g, for 10 min) and resuspension in fresh buffer. 400 μl of Leydig cell suspension (0.4 – 1 x 10^4 cells/ml) was mixed with the 200 μl of metal solution including 3 nM LH and incubated in ahaking incubator under 5 % CO_2: 95 % O_2 at 37 °C for 2 hours. - Preparation of adrenal cells and treatments Adrenal glands were removed from male SD rats (400 – 450 g) and decapsulated to collect mainly fasciculata and reticular cells. The decapsulated gland was minced and treated with 3 mg/ml of collagenase in shaking incubator for 1 hour. The supernatant including dissociated cells were collected and then washed through repeated centrifugation (800 g for 10 min). The final cell suspension of adrenal cells were adjusted to 10^4 cells/ml in Krebs-Ringer bicarbonate buffer containing 1 mg/ml of glucose. 225 μl of cell suspension was mixed with 25 μl of metal solution (10^{-6}, 10^{-5}, 10^{-4}) including 10 nM ACTH and incubated in shaking incubator under 5 % CO_2: 95 % O_2 at 37 °C for 2 hours. - Determination of steroids The corticosterone and testosterone was assayed by radioimmunoassay.
Year	:	1990
GLP	:	No
Dose levels	:	10^{-6}, 10^{-5}, 10^{-4} M
Exposure period	:	The isolated cells were exposed to metal solution for 2 hours.
Frequency of treatment	:	Single treatment
Test substance	:	Other TS: Iron dichloride, purity = 98 %, Sigma-Aldrich, Lot No. – 23828CB

Test conditions	:	- Source of the cells Leydig cells were isolated from the rat. However the strain was not specified. Adrenal cells were isolated from the SD rats. - The results were examined by student's t test.
Results	:	- Iron dichloride did not affect the corticosterone production of Leydig cells and the testosetone production of adrenal cells at 10^{-6}, 10^{-5} and 10^{-4} M.
Conclusions	:	Iron dichloride showed no direct toxic effects on steroid production by Leydig and adrenal cells. There is no effect on the viability of the cells under the test conditions.
Reliability	:	(3) Not reliable
Reference	:	(56)

5.9 DEVELOPMENTAL TOXICITY / TERATOGENICITY

Species/strains	:	Rat (Sprague-Dawley)
Sex	:	Male/Female
Route of administration	:	Oral (Gavage)
Method	:	OECD TG 422 "Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test"
Year	:	2004
GLP	:	Yes
Dose levels	:	0, 125, 250 and 500 mg/kg b.w./day
Exposure period	:	42 days for male animals and 42 to 54 days for female animals
Frequency of treatment	:	Daily
Control groups	:	Yes (Concurrent no treatment)
Statistical methods	:	Same as for "repeated dose toxicity (OECD TG 422)".
NOAEL for developmental toxicity	:	500 mg/kg b.w./day
Test conditions	:	**Test organism** - Sex: male/female - Age of animals: 8 weeks old for males and females - Weights during the repeated dose toxicity study: 269.23 – 302.18 g for males and 191.34 – 221.60 g for females - Number of test animals: 70 animals for each sex **Observation of F1** - Birth rate and survival rate - Body weight and crown rump length(CRL): measured on the day 0 and 4 at postpartum - Sex ratio - External findings in neonates
Test substance	:	Other TS: Iron dichloride purity = 98 %, Sigma-Aldrich Corporation, LOT No. – 14330TA
Results	:	**Results for F1** - *Litter size, birth rate, survival rate, and sex rate*: All data were within the normal range. - *Body weight and crown rump length(CRL) of neonates*: CRL of neonates were significantly decreased as compared with that of the control group for postpartum day 4 in 125 mg/kg b.w./day treatment group ($p < 0.01$). But the decrease did not correlate with body weights that is a main growth and developmental index. Since there was no dose-dependence of CRL decrease, it was concluded that the test substance did not influence the growth of neonates. - *External findings in neonates*: A case of acaudate was observed at 500 mg/kg b.w./day treatment group. However, because of the low frequency of occurrence, it was not a teratogenic effect

Table. Litter size, birth rate, survival rate, and sex ratio

DOSE: (mg/kg)	0	125	250	500
Mean litter size	14.2	14.3	14.4	14.6
Birth rate (%)	96.8	100	98.0	97.3
Postpartum 0 day's survival rate (%)	96.8	100	98.0	97.3
Postpartum 4 day's survival rate (%)	97.4	98.8	98.5	98.6
Postpartum 0 day's sex ratio (%)	1.0	1.3	1.1	0.9
Postpartum 4 day's sex ratio (%)	1.0	1.3	1.1	0.8

Table. Body weight(B.W.) and Crown rump length(CRL) of neonates

Sex of neonates		Male				Female			
Group/ Dose(mg/kg)		G1 0	G2 125	G3 250	G4 500	G1 0	G2 125	G3 250	G4 500
B.W. (g) (postpartum day 0)	Mean	7.13	7.04	7.21	6.99	6.70	6.63	6.61	6.69
B.W. (g) (postpartum day 4)	Mean	11.50	10.87	11.60	10.62	10.84	10.39	10.65	10.05
CRL (cm) (postpartum day 0)	Mean	4.17	4.27	4.34	4.27	4.11	4.31	4.20	4.16
CRL (cm) (postpartum day 4)	Mean	5.29	5.09	5.28	5.22	5.10	4.86*	5.13	5.01

*: Statistical significance was observed.

Table. External findings in neonates

Organs	Findings	Group/ Dose (mg/kg)	postpartum day 0				postpartum day 4			
			G1/ 0	G2/ 125	G3/ 250	G4/ 500	G1/ 0	G2/ 125	G3/ 250	G4/ 500
		No. of neonates examined	151	172	197	142	147	170	194	140
Tails	Acaudate	No. of neonates founded	0	0	0	1	0	0	0	1

0: No abnormalities detected
1: One case of Acaudate was detected

Reliability : (1) Reliable without restrictions
Flag : Critical study for SIDS endpoint
Reference : (42)

5.10 OTHER RELEVANT INFORMATION

A. SPECIFIC TOXICITIES (NEUROTOXICITY, IMMUNOTOXICITY ETC.)

B. TOXICODYNAMICS, TOXICOKINETICS

Type : Toxicokinetics

Remarks : The absorption of iron is regulated by a complex mechanism to maintain homeostasis, mainly involving intake, stores, and loss. Generally, about 2 to 15 percent is absorbed from the gastrointestinal tract, whereas elimination of absorbed iron, is only anout 0.01 % per day. During periods of increased iron need (childhood, pregnancy, or blood loss), absorption of iron is greatly increased. Absorption is occurs in two steps. Absorption of ferrous ions from the intestinal lumen into the mucosal cells and transfer from the mucosal cell to plasma, where it is bound to transferrin for transfer to storage sites. Transferrin is produced in the liver. As ferrous iron is released into plasma, it becomes oxidized by oxygen in the presence of ferroxidase I. There are 3 to 5 g of iron in the body. About two-thirds is bound to hemoglobin, 10 % in myoglobin and iron-containing enzymes, and the remainder is bound to the iron storage proteins ferritin and hemsiderin. Exposure to iron induces synthesis of apoferritin, which then binds ferrous ions. Iron may be released form ferritin by reducing agents. Normally, excess ingested iron is excreted, and some is contained whithin shed intestinal cells and in bile and urine and in even smaller amounts in sweat, nails, and hair. Total iron excretion is usually on the order of 0.5 mg/day.
Reliability : (4) Not assignable
Reference : (57)

Type : Toxicokinetics
Remark : The oral absorption of iron is largely limited by physiological homeostatic mechanisms that regulate the intake based on need. The intestinal mucosa is the major site at which the absorption is limited, but hepatic and pancreatic secretions may influence the absorption. However, in cases of acute iron poisoning, the gastric mucosa is often disrupted. The iron transport system is overloaded, and this results in circulating free iron. In the normal homeostatic mode the divalent iron is absorbed into the gastric mucosa, where it is converted to the trivalent form. The trivalent iron attached to ferritin passes into the bloodstream and is converted into transferrin. Transferrin is transported to the spleen or liver, where it is stored as ferritin or homeosiderin. Under normal conditions the body burden of iron is about 4 g. Hemoglobin contains the greatest amounts of body iron (67 %), and this is largely in the red blood cells. Twenty-seven percent of the total body iron is in the liver as ferritin or in pathological conditions as hemosiderin. Because iron is so important in physiological function, the body tends to conserve iron. The major mechanisms for the excretion of iron are desquamation of the gastrointestinal tract and blood loss. However, the iron-defoxamine formed as the result of administering the specific iron chelator, deferoxamine, is excreted in urine.
Reliability : (4) Not assignable
Reference : (58)

Type : Toxicokinetics
Remarks : Specific Metal-Binding Proteins
Transferrin is a glycoprotein that binds most of ferric iron in plasma. Transport of iron across cell membrane occurs by receptor-mediated endocytosis of ferric transferrin. The receptor is a disulfied-linked membrane glycoprotein whose affinity for apotransferrin is two orders of magnitude lower than that for ferric transferrin. Once inside the cell, iron is separated from transferrin by an acidification process within the endosomes. This protein also transports Al^{3+} and Mn^{2+}.
Ferritin is primarily a storage protein for iron in the reticuloendothelial cells of liver, spleen, and bone. It plays a major role in hepatic turnover of iron. Kupffer cells release iron acquired from the phagocytosis of red blood cells in the form of ferritin, which is efficiently internalized by hepatocytes via their ferritin receptors. It has been suggested that ferritin may serve as a

general metal detoxicant, since it binds a variety of toxic metals including Cd, Zn, Be and Al.
Reliability : (4) Not assignable
Reference : (57)

5.11 EXPERIENCE WITH HUMAN EXPOSURE

Type : Human poisoning
Results : For toxicology purposes, estimates of ingested iron dose are based on the amount of elemental iron in the particular iron complex. Iron dichloride contains 28 % of iron element. Although a single vaule of the toxic dose has not been established, significant gastrointestinal manigestations occur following the ingestion of 20 mg of elemental iron per kilogram of body weight while systemic toxicity may occur following the ingestion of at least 60 mg of elemental iron per kilogram of body weight.
Reliability : (4) Not assignable
Reference : (59)

Type : Human poisonings
Results : *Gastrointestinal toxicity:* Iron acutely and directly damages the gastrointestinal tract. These effects include vomiting, rapid onset of diarrhea, colicky abdominal pain, and gastrointestinal hemorrhage.
Circulatory shock: Direct addition of iron in the ferrous state to blood may directly decrease bicarbonate. The inhibitory effects of iron on complex oxidative metabolism at cellular level may also result in increasing lactic acid concentration. Low cardiac output and high systemic resistance are the clinical presentations seen in the acutely iron-intoxicated infant. Such infants are tachycardic and extremely pale, with cold extremities, and central venous pressures are decreased.
Hepatic necrosis: The liver is a target organ for damage by direct uptake of iron. It appears that iron can be taken up directly by the reticuloendothelial system of the liver and spleen and that it exerts a toxic effect on the mitochondria of the liver cell.
Gastic scarring: Following an acute corrosive insult to the gastrointestinal tract, the healing process may result in areas of stenosis in both the stomach outlet and the small bowel. These late consequences of iron poisoning occur rarely. They may present as late as 2 to 6 weeks after the inciting event.
Reliability : (4) Not assignable
References : (60)

Type : Acute human poisoning
Results : *Mechanism of iron toxicity:* Generally, doses of various ferrous of ferric salts greater than 150 mg/kg of elemental iron are considered serious. Significant concentrations of either the ferrous or ferric form of iron lead to rapid necrosis of the mucosa of the gastrointestinal tract and subsequent severe hemorrhage. Concomitantly, absorbed iron soon exceeds the binding capacity of transferrin, and serum iron concentration rise rapidly. When serum laden with iron passes through the liver, the liver sequesters sizable amounts, as does the spleen. A severe metabolic acidosis rapidly and steadily ensure. Moreover, the clinical picture of shock far in excess of that expected from blood loss alone follows. (Conceivably ferritin from mucosal cells or the liver may be serving as the responsible vasodepressor) In addition, an elevated serum iron interferes with the clotting mechanism augmenting the hemorrhagic process.
Clinical manifestations: Symptoms of iron poisoning begin with vomiting, often accompanied by abdominal pain and diarrhea. Caused by the

irritation of iron, it ensues from 30 minutes to 2 hours after ingestion. Almost immediately, a state of shock may become apparent. Shock is belived to stem from a combination of gastrointestinal – hemorrhage and extracellular fluid loss into gastrointestinal tract – and incompletely understood systemic factors. Liver failure then becomes obvious, carbohydrate metabolism is drastically impeded, and a deepening coma follows.

Reliability : (4) Not assignable
References : (61)

Type : Case study
Species : Human
Method : Other
Year : 1980
Test substance : Other TS: The solution is the waste product from plating copper on iron electrodes.
Results : The patient expired on his fifth hospital day.
Remarks : Case conditions
- *age/race/sex*: 18 years old/white/male
- *Route of intoxication*: Oral ingestion, aspiration, and absorption through burned skin
- *Exposure solution*: a composition of saturated iron dichloride (0.9 molar) in dilute hydrochloric acid (0.03 normal) with a small but variable amount of copper (2000 parts per million)
- *Exposure period*: after falling into a vat of iron dichloride solution, the subject jumped out of the vat immediately.

Case report
- *1 st day*: improvement of all parameters
- *2 nd day*: condition deteriorated (progressive acidosis associated with hypoxia and ventilatory failure which required intubation and mechanical support; rising bilirubine level; development of ketoacidosis)
- *3 th day*: development of intravascular coagulopathy with a bleeding diathesis which was treated with heparin and intravenous clotting factors.
- *4 th day*: starting iron clearing treatments with intravenous deferoxamine after figuring out the iron content of the vet and hemodialysis because of total anuric which became shortly after starting deferoxamine treatment.
- *5 th day*: condition deteriorated and the patient expired

Post-mortem examination findings
- *Oropharyns and esophagus*: hyperemic area but no areas of erosion; mcroscopic examination: superficial mucosal necrosis in the epithelium with the sign of regeneration
- *Stomach*: superficial hemorrhagic areas on the serosa and clotted blood with multiple mucosal erosions; mcroscopic examination: massive submucosal hemorrhage and large amount of iron in the mucosa
- *Jejunum and ileum*: hyperemic and with iron demonstration
- *Colon*: normal
- *Liver*: large(2625 g) and with areas of patchy, fatty and hemorrhagic necrosis; microscopic examination: no involvement of any hepatic lobules, preserved portal triads, evident increase in proliferation in bile duct and bile stasis in preserved parenchyma; increased iron contents in liver, especially in the reticuloendothelial system.
- *Larynx, trachea, and mainstem bronchi*: hemorrhagic erosions covered with a green film; mcroscopic examination: a denudation of the epithelium; high iron content in mucosa, submucosa and cartilage, demonstrated by Prussian blue staining
- *Lung*: parenchyma of the lungs with bronchopneumonia, associated with hemorrhagic necrosis, congestion, and foci of hyaline membrane
- *Heart*: grossly normal; Microscopic examination: microthrombi
- *Kidney*: large (right 225 g and left 200 g) with pale cortices and

hyperemic medullae; mcroscopic examination: red cell dasts and hydropic changes of the tubular epithelium, hemoglobin casts of the distal tubules and normal glomeruli

Conclusions : Iron intoxication by aspiration and absorption through lungs and skin caused to develop acute iron intoxication symptoms in stage and then finally to die.

Reliability :

Reference : (62)

Type : Acute human poisoning
Species : Human
Method : Other
Year : 1964
Test substance : Other TS: Ferros sulfate
Results : One case of death from acute iron poisoning despite early treatment with ethylenediaminetetra-acetic acid (EDTA), and two cases in which a successful outcome followed the use of desferrioxamine and diethylenetriamine-penta-acetic acid (DTPA) respectively.

Remarks : ***Case Conditions***

Case 1:

- *age /sex/body weight:* 17 months old/male/14-5kg
- *Route of intoxication*: oral ingestion
- *Exposure ingesting*: more than 100 ferrous sulphate tablets, that is, 20 g of ferrous sulphate, or 7.3 g of elemental iron.
- *Exposure period*: after ingesting, a boy was admitted to hospital one hour later.

Case 2:

- *age /sex/body weight:* 22 months old/female/12kg
- *Route of intoxication*: oral ingestion
- *Exposure ingesting*: about 13 g of ferros sulphate, or 4.7 g of elemental iron
- *Exposure period*: a girl arrived at hospital half an hour later.

Case 3*:*

- *age /sex:* 20 years old/female (10 weeks pregnant)
- *Route of intoxication*: oral ingestion
- *Exposure ingesting*: with suicidal intent 60 ferros sulphate tablets (12.8 g of ferrous sulphate or 4.6 g of elemental iron) together with 1.5 g of sodium amytal and 6 g of aspirin.
- *Exposure period*: She was seen one and a half hours after ingestion.

Case Reports

Case 1: - Post exposure treatment*:* A weak solution of sodium bicarbonate and milk was given through the stomach tube. A saphenous vein cut-down was performed, and 25 g of albumin was rapidly infused intravenouly. This was followed by a glucose-saline solution containing 300 mg, EDTA per 500 ml, which was infued at the rate of 50 ml/hr. A total of 1.2 g of EDTA was given in the first 24 hours. The gastric lavage was completed with weak sodium bicarbonate solution, 500 mg of desferrioxamine was given through the stomach tube. A further 500 mg of desferrioxamine together with an aluminium hydroxide mixture were introduced through the indwelling gastric tube every four hours. A total of 1.2 g of desferrioxamine was given intravenously over the first 48 hours.
- Clinical signs*:* On arrival, he was drowsy and pale. With gastric lavage, dark brown fluid containing undigested tablets was returned. Withing 30 minutes of commencement of the EDTA infusion, he was easily roused, showed some interest in his surroundings, but remained lethargic. He had frequent blood stained motions and vomitted small quantities of red blood.
- Autopsy examination**:** The pharynx, larynx and trachea appeared

normal. Both lungs were congested, with a few potechial haemorrhages on the pleural surfaces. There were numerous large haemorrhages scattered over the pericardium on both anterio and posterior aspects. The stomach contained a small quantity of brownish fluid its mucosa was thickened, brownish and of leathery consistency and the peritoneal surface was haemorrhagic. The liver was enlarged and was yellowish in color. The spleen was firm and dark in color, the kidneys showed congestion of the pyramids but were otherwise normal. The other organs appeared normal.

Case 2:
- Post exposure treatment: An intravenous infusion was commenced and 25 g of albumin and 300 ml of normal saline were infused rapidly.
- Clinical signs*:* The child began to vomit almost immediately and arrived at hospital half an hour later. During gastric lavage she rapidly developed peripheral circulatory failure and lost consciousness. On the second day after admission, the liver enlarged and increased to a maximum of 5 cm below the right costal margin on the sixth day. Liver fuction tests showed evidence of hepatocellular damage.
The child's clinical state improved progressively and on the 10^{th} day, when she was discharged, liver funtion tests gave normal results and she appeared very well.

Case 3*:*
- Post exposure treatment and Clinical signs*:* After an intravenous infusion of glucose-saline and albumin, the blood *pressure* was 115 mmHg. A gastric lavage performed, and numerous undigested white particles were recovered. 1 g of DTPA 465 µg/100 ml in 500 ml of glucose-saline was infused intravenously over the next hour. After the infusion, the patient's blood pressure, color and state of cosciousness improved and remained satisfactory. Within 24 hours of admission, her mental state was normal.
The urinary excretion of iron during the six hours after the start of the infusion of DTPA was 2200 µg, which is 10 times the excretion after the infusion of 1 g of DTPA in normal subjects.

Conclusions : DTPA given intravenously in a dose of 25 to 50 mg/kg/day may be substituted.
Reliability : (2) Reliable with restrictions
2e: Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag : Critical study for SIDS endpoint
Reference : (63)

Type : Acute human poisoning
Species : Human
Method : Other
Year : 1974
Test substance : Other TS: Ferros succinate
Results : Two adult patients were successfully treated, one without complications and the other with circulatory failure, hepatic involvement and hemolysis.
Remarks : ***Case Conditions***
Case 1:
- Age /sex/: 19 years old/female
- Route of intoxication: Oral ingestion
- Exposure ingesting: 60 tablets of ferrous succinate, that is, 2 g Fe^{++} or 42 mg Fe^{++}/kg bw.
- Exposure period: After ingesting, she was admitted to hospital 2.5 hour later.

Case 2:
- age /sex: 26 years old/female (mother of three children)

- *Route of intoxication*: oral ingestion
- *Exposure ingesting*: 150 tablets of ferrous succinate, corresponding to 11g Fe^{++} or 180 mg Fe^{++}/kg bw.
- *Exposure period*: The tablets were ingested during 3 hours prior to admission.

Case Reports

Case 1:

- *Post exposure treatment:* Immediately after admission vomiting was elicited. One hour later she was given 1 g desferrioxamine i.m. and then the same amount 3 hours later.
- *Clinical features:* Differential count such as Hb, plasma iron, TIBC, WBC, urine sediment, serum electrolytes and liver tests showed normal values.

: ***Case 2:***

- *Post exposure treatment*: Treatment with desferrioxamine, 1 g i.m., was initiated 50 min after admission. When a ventricular tube was inserted, profuse vomiting was elicited. On the first day the patient was given desferrioxamine, 1 g i.m., 1 g i.v. and 5 g per as via the ventricular tube. On the second day she received 2.5 g i.v. and 5 g per as via the tube, and after that 1 g×4 i.m. during one week.
- *Clinical features:*

-The 1st day: She felt sick and vomited frequently. The volum of the vomitus was estimated to be about 6 L. The patient became hypovolemic with tachycardia and falling blood pressure. Temperature increased to a maximum of 38.2□ after 12 hours, and the patient remained subfebrile for one week.

- The 2nd day: She became icteric and polyuric. The liver tests showed pathological values, culminating. Hemolysis was indicated by the observation of free Hb in plasma and urine, depletion of haptoglobin and a slight thrombocyte depression. A slight hypokalemia was compensated. The patient was checked one month after admission. She felt well and the results of routine laboratory tests were normal.

Conclusions : Iron intoxication by ingesting is evidence of intestinal bleeding, positive test for blood in the stools of one and hematemesis in the other.

Reliability : (2) Reliable with restrictions
2e: Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

Reference : (64)

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