FOREWORD INTRODUCTION

CHOLINE CHLORIDE

CAS N°: 67-48-1

SIDS Initial Assessment Report

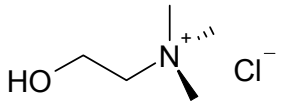
For

SIAM 19

Berlin, Germany, 19-22 October 2004

1. Chemical Name:	Choline chloride
2. CAS Number:	67-48-1
3. Sponsor Country:	United Kingdom Dr Steve Robertson Environment Agency National Centre for Ecotoxicology & Hazardous Substances Isis House, Howbery Park, Wallingford OX10 8BD, UK Fax: +44 1491 828 556 e-mail: steve.robertson@environment-agency.gov.uk
4. Shared Partnership with:	BASF AG, Germany; Air Products Chemicals, The Netherlands; Taminco NV, Belgium
5. Roles/Responsibilities of the Partners:	
• Name of industry sponsor /consortium	Akzo Nobel Chemicals NV Chris Braun Stationsplein 4 3800 AE Amersfoort The Netherlands
• Process used	
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals Programme ?	This substance is sponsored by the UK under the ICCA Initiative and is submitted for first discussion at SIAM 19
7. Review Process Prior to the SIAM:	The industry consortium collected new data and prepared the updated IUCLID, and draft versions of the SIAR and SIAP. UK government peer-reviewed the documents and audited selected studies.
8. Quality check process:	
9. Date of Submission:	23 July 3004
10. Date of last Update:	
11. Comments:	

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	67-48-1
Chemical Name	Choline chloride
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Category/Analogue Rationale

In some circumstances, available data for other choline salts (e.g. choline magnesium salicylate) have been evaluated in Human Health to assist the weight of evidence approach for choline chloride. Due consideration was given to potential toxicity exerted by byproducts e.g. in parenteral exposure.

Human Health

Choline is a dietary component and found in foods as free choline and as esterified forms such as phosphocholine, glycerophosphocholine, sphingomyeline, and phosphatidylcholine. It functions as a precursor for acetylcholine, phospholipids, and the methyl donor betaine and is important for the structural integrity of cell membranes, methyl metabolism, cholinergic neurotransmission, transmembrane signaling, and lipid and cholesterol transport and metabolism.

Dietary choline is absorbed from the lumen of the small intestine. Additionally to dietary supply choline can be made available by enzymatic cleavage in the pancreas from other nutritional sources (e.g. phosphatidylcholine). Before choline can be absorbed from the gut, some is metabolised by bacteria to form betaine and methylamines. Fasting plasma choline concentrations vary from 9 to 20 µmol/L.

The critical adverse effect from high intake of choline is hypotension, with corroborative evidence on cholinergic side effects (e.g., sweating and diarrhoea) and fishy body odour. After inadequate dietary intake decreased choline stores and liver damage (as assessed by elevated alanine aminotransferase) may develop.

Animal studies with choline chloride show a low acute toxicity after oral uptake (with a range of LD50s of 3150 – ≥5000 mg/kg bw determined in different studies). . No acute toxicity attributable to choline was observed in humans following oral doses of ≥3000 mg choline magnesium trisalicylate/day.

In rabbits, choline chloride may lead to a slight irritation of the skin and eye. No data on sensitization in animals are available. The skin sensitisation potential of choline chloride is regarded as negligible in humans.

In a limited, specialised, repeated dose study designed to investigate the impact of choline on the liver tumour promoting activity of phenobarbital and DDT in DEN-initiated animals, rats were dosed with approximately 500mg/kg bw/day over 72 weeks via feed, with a post-observation period of 30 weeks. No significant effects were observed relative to controls with respect to survival rates, body weights and relative liver weights. Only limited pathological investigations were carried out at autopsy (gross examination with histological investigation of only the liver and any tissues showing gross abnormalities). No adverse effects were observed. Therefore the NOAEL for this study was ≥500mg/kg bw/day. The tolerable upper intake level for human adults was set at 3.5 g/day corresponding to approx. 58 mg/kg bw/day (USA's Standing Committee on the Scientific Evaluation of Dietary Reference Intakes).

Choline chloride does not show a mutagenic, clastogenic or DNA damaging potential when tested *in vitro*; furthermore it has no structural alerts. There is therefore no indication of a genotoxic potential *in vivo*.

No developmental toxic effects were observed in mice after oral doses of 1250 mg/kg bw/day on gestation days 1 to 18. Doses above the levels recommended currently (4160 mg/kg bw/day and higher) and associated with maternal toxicity, did produce developmental toxic effects, but these were secondary to the maternal toxicity at the excessive doses used. The compound does not produce any significant developmental toxicity in the mouse.

Thus evidence from animal studies and from human exposure indicates that choline chloride has low toxicity, is not mutagenic and has no developmental toxicity. This is not unexpected in view of its presence in the diet and its production in metabolic processes in the body; it fulfils key roles in nerve transmission, cell membrane integrity, and lipid metabolism. Only limited animal data are available on effects on fertility, but the normal exposure of humans to appreciable amounts of choline chloride both from the diet and formed from normal metabolic processes, would argue against it having any significant adverse effects on fertility. This is supported by the fact that it has been widely used as an animal feed additive for decades with no apparent adverse effects being noted on fertility.

Environment

Choline chloride is a white crystalline solid but is marketed as an aqueous solution (70 – 75 % w/w in water) which is a colorless liquid with an amine-like odor. It has a measured water solubility of ca. 650 g/L (calculated water solubility: 1,000,000 mg/L) and a calculated vapor pressure of $6.57*10^{-10}$ hPa at 25°C. A Henry's Law Constant of $2.06*^{10-}11$ Pa*m³/mole at 25 °C could be calculated. Distribution modeling using Mackay Level I indicates water (100 %) to be the main target compartment. The amount in the other compartments is with < 0.0001 % negligible. Choline chloride is readily biodegradable according to OECD-criteria (MITI-I Test; BOD measurements) reaching 93 % degradation within 14 days. Due to the chemical structure hydrolysis can be excluded. In the atmosphere choline chloride will be rapidly degraded according to a half life time ($t_{½}$) of about 6.9 hours for hydroxyl-radicals based on a 12 hours day. Due to the measured and calculated $logK_{ow}$ of –3.77 and –5.16 both at 25°C, respectively, and a calculated $logK_{oc}$ of 0.37 a bio- or geoaccumulation is not to be expected.

The aquatic toxicity has been determined for freshwater and saltwater species according to several GLP and non-GLP test guidelines. For the freshwater fish species *O. latipes* a LC_{50} (96h) of > 100 mg/L and for the saltwater fish species *L. limanda* a LC_{50}(96h) of > 1,000 mg/L could be determined, respectively. The acute toxicity (EC_{50}) for the invertebrate species *D. magna* was found to be 349 mg/L after 48 h of exposure, In a 21d Daphnia reproduction test, a 21d NOEC (reproduction) of 30.2 mg/l was obtained. For the freshwater algae *Pseudokirchneriella subcapitata* an $Er(b)C_{50}$ (72h) of > 1,000 mg/L could be determined.

Exposure

The world production of choline chloride in the year 2002 was in the range of10,000 to 50,000 tons.

At the European production sites choline chloride is produced under pressure and room temperature by reaction of trimethylammonium chloride with ethylene oxide in closed systems. Exposure may occur during manufacture, transportation and industrial use. The likely primary routes of human exposure to choline chloride are skin contact and inhalation at the work place. Worker exposure is limited by enclosed systems, industrial hygiene controls and personal protective measures are adequate.

Choline chloride has a widespread use as a food additive for animal husbandry since the early 1930s. For this application area almost 100% of the produced choline chloride is either premixed as solid and then directly mixed with animal feed or marketed as a fluid compound to the customers and directly released into special installed mixing apparatus. A very small amount of the choline chloride production is used for formulations in the field of plant growth regulators. In general the following formulations are used for commercial applications: Bulk, in solution (up to 70%), on vegetable carriers, on amorphous silica carriers.

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

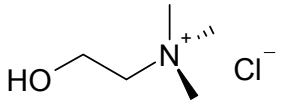
The chemical is currently of low priority for further work due to its low hazard profile.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 67-48-1
IUPAC Name: Ethanaminium, 2-hydroxy-N,N,N-trimethyl-, chloride
Molecular Formula: $C_5H_{14}NO.Cl$
Structural Formula:



Molecular Weight: 139.63 g/mole
Synonyms: (2-Hydroxyethyl)trimethylammonium chloride
(β-Hydroxyethyl) trimethylammonium chloride
ammonium, (2- Hydroxyethyl)trimethyl-, chloride
Bilineurin chloride
Biocoline
Choline hydrochloride
Cholinium chloride
Ethanaminium, 2-hydroxy-N,N,N-trimethyl-, chloride
Hepacholine
Hormocline
Lipotril
Luridin chloride
Neocolina
Paresan
Trimethyl(2- hydroxyethyl) ammonium chloride

1.2 Purity/Impurities/Additives

Trimethylamine:	max. 500 ppm
Ethylene glycol:	max. 500 ppm
Organic purities (TMA+glycol+chloroetheanol):	max. 1500 ppm
Colour:	max. 50 hazen
Heavy metals as lead:	max. 20 ppm

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Remarks and Citations
Physical state	white crystalline solid Liquid, colorless, amine-like odor	pure choline chloride Solution 75 % w/w in water; BASF AG (2002) (Data refer only to Safety Data Sheet information)
Melting point	247°C	The information corresponds to the pure substance; ICSC 0853 (2004)
Boiling point	not applicable due to decomposition on heating	
Relative density	1.1g/cm^3 at 20°C	70 ± 1 % choline chloride, 30 % water, less than 0.05 % impurities measured; BASF AG (1974) Data refer to a technical data overview, no details of method used and year the study was performed is given. Further, as the value refers to a solution the density of the pure substance may differ.
Viscosity	21 mPa*s at 20°C	70 ± 1 % choline chloride, 30 % water, less than 0.05 % impurities; measured; BASF AG (1974) Data refer to a technical data overview, no details of method used and year the study was performed is given.
Vapor pressure	0.0000000657 Pa at 25°C	Calculated using MPBPWIN v1.40, refers to the pure substance; BASF AG (2003a)
Water solubility	ca. 650 g/l	50 % choline chloride powder; pH = 6 – 7; measured; BASF AG (1974): Study reliability was not assignable. A calculated value for the pure substance of 1000 g/l was estimated using WSKOW v1.40; BASF AG (2003b)
Partition coefficient n-octanol/water (logK_{ow})	-3.77 at 25°C	Solution 75 % w/w in water; measured; BASF AG (1988a)
Henry's law constant	2.06*10E-11 Pa*m³/mole at 25°C	Calculated using HENRYWIN v3.10 (bond method), refers to the pure substance; BASF AG (2003d)
logK_{oc}	0.37	Calculated using the PCKOCWIN v1.66 calculation program (K_{oc} = 2.3), refers to the pure substance; BASF AG (2003e)

Choline chloride is a quaternary amine salt, it dissociates in water into the corresponding positively charged quaternary hydroxyl alkylammonium ion and the negatively charged chloride ion. Data using different amounts of choline chloride show that the lowest pH value of 4 was determined at 100 mg/L. Even if choline chloride of different specifications was used, choline chloride can be stated to be a weak acid. No data on dissociation constants are available for this compound.

Choline chloride has neither explosive nor oxidizing properties due to its molecular structure (BASF AG, 1999).

1.4 Analog Justification

In some circumstances, available data for other choline salts (e.g. choline magnesium salicylate) have been evaluated in Human Health to assist the weight of evidence approach for choline chloride. Due consideration was given to potential toxicity exerted by byproducts e.g. in parenteral exposure.

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

The world production of choline chloride in the year 1984 was about 85,000 tons (Ullmann's Encyclopedia, 2000). In the year 2002 the world production of choline chloride was in the range of 10,000 to 50,000 tons.

According to the data cited in the SPIN database choline chloride was used in non-consumer preparations with 11 tonnes in 1999 in Sweden, and in the year 2000 in Sweden and Denmark with 34 and 29.8 tonnes, respectively (http://www.spin2000.net/spin.html).

At the European production sites choline chloride is produced under pressure and room temperature by reaction of trimethylammonium chloride with ethylene oxide in closed systems. The reaction product choline chloride is free of ethylene oxide because it is entirely consumed in the production process.

Choline chloride has had wide dispersive use as a food additive for animal husbandry since the early 1930s. For this application area almost 100% of the produced choline chloride is either premixed as solid and then directly mixed with animal feed or marketed as a fluid compound to the customers and directly released into special installed mixing apparatus. A very small amount of the choline chloride production is used for formulations in the field of plant growth regulators. In general the following formulations are used for commercial applications: bulk, in solution (up to 70%), on vegetable carriers, on amorphous silica carriers.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Releases into the environment may occur during production and processing of choline chloride, as well as from the use of the substance or products containing it.

Via production and further processing in 2000 less than 5 kg/a of choline chloride were emitted into the air according to the German Emission Register at BASF AG in Ludwigshafen (Germany)(BASF AG, 2003f).

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

No data of choline chloride in the influent or effluent of wastewater treatment plants or in surface waters are available so far.

Choline chloride is the salt of the naturally occurring choline, the pre-stage of the neurotransmitter acetylcholine, which is important for mnemonic and thought-processes. Choline occurs naturally in fungi, hop and kingcups and as integral part of lecithin. Choline chloride is a common food additive in animal husbandry.

2.2.2 Photodegradation

In the air the substance will be rapidly degraded according to the calculated $t_{1/2}$ of about 6.9 hours for OH-radicals using the model AOP v1.90 on the basis of a 12 hours day (BASF AG, 2004a).

2.2.3 Stability in Water

Choline chloride is a quaternary ammonium salt and dissociates in water. No measured data on the stability of choline chloride in water are available. Hydrolysis at environmental pH conditions is not to be expected due to the chemical structure of choline chloride.

2.2.4 Transport between Environmental Compartments

Releases into the environment may occur during production, further processing and use of choline chloride. Distribution modelling using Mackay Level I V2.11 indicates water to be the main target compartment with 100 % for the pure choline chloride (BASF AG, 2004b). The amounts of choline chloride in the other compartments air, soil, sediment, suspended sediments, fish and aerosol is negligible (< 0.0001%).

The estimated $\log K_{oc}$ using the PCKOCWIN model was 0.37 (BASF AG, 2003d). This indicates that choline chloride will not adsorb on soil and sediments or suspended solids.

The calculated Henry's Law Constant using the HENRYWIN model (bond method) was $2.06*10^{-11}$ Pa*m³/mole (BASF AG, 2003 c). This indicates that choline chloride will not rapidly evaporate into the atmosphere.

2.2.5 Biodegradation

Choline chloride was shown to be readily biodegradable according to OECD-criteria (93 % biodegradation within 14 days) in a MITI I-Test (MITI, 1992). The biodegradation was recorded by measuring the BOD. This result can be confirmed by Tunkel *et al*. (2000) who stated that a biodegradation of ≥ 60 % in a MITI-I test corresponds to a ready biodegradation. In addition, in a BOD_5 test performed according to DIN 38409 part 43 a $BOD_5/ThOD_5$ ratio of 75 % obtained, which also confirms a ready biodegradation of choline chloride (BASF AG, 1984).

2.2.6 Bioaccumulation

No measured data on bioaccumulation are available. Based on the partition coefficient octanol-water (measured $\log K_{ow}$ = -3.77), and the BCF estimation using the equation cited in the TGD (2003): $\log BCF = 0.85*\log K_{ow} - 0.70$ a BCF of 0.59 can be derived and therefore, bioaccumulation is not expected in aquatic organisms. In contrast, using the model BCF v2.14 based on the calculated $\log K_{ow}$ of –5.155 a BCF of 3.16 can be derived (BASF AG, 2003f). Both values differ by a factor of 6. Nevertheless, the bioaccumulation potential of choline chloride can be stated to be low.

2.2.7 Other Information on Environmental Fate

No other information is available.

2.3 Human Exposure

Choline is a dietary component that is important for the structural integrity of cell membranes, methylation metabolism, cholingeric neurotransmission, transmembrane signalling, and lipid and cholesterol transport and metabolism. Human cells grown in culture have an absolute requirement for choline. When cells are deprived of choline, they die by apoptosis. There is an endogenous pathway for the *de novo* biosynthesis of the choline moiety via the sequential methylation of phosphatidylethanolamine using S-adenosylmethionine as the methyl donor. Thus, the demand for dietary choline is modified by metabolic methyl-exchange relationships between choline and three nutrients: methionine, folate, and vitamin B12. Choline occurs naturally in fungi, hop and kingcups and as integral part of lecithin. It occurs in many components of the diet, both as free choline and as phosphatidylcholines, such as lecithin, e.g. in egg yolk, vegetables and animal fat (Standing Committee on the Scientific Evaluation of Dietary Reference Intake, 2000).

Choline is thus an essential component in the body for normal health. There is debate about whether it is an essential component of the diet, due to the *de novo* synthesis in the body. However, there is evidence to indicate that such synthesis is not always sufficient to meet human requirements (Standing Committee on the Scientific Evaluation of Dietary Reference Intake, 2000)

2.3.1 Occupational Exposure

Exposure may occur during manufacture, transportation and industrial use. The likely primary routes of human exposure to choline chloride are skin contact and inhalation at the work place. Worker exposure is limited by enclosed systems, industrial hygiene controls and personal protective measures (protective gloves, safety glasses with side-shields, respiratory protection if ventilation is inadequate).

2.3.2 Consumer Exposure

As noted above, choline and phosphatidylcholines, such as lecithin, are widely distributed in food. Estimated average choline dietary intake in adults consuming typical U.S. or Canadian diet (as free choline and the choline in phosphatidylcholine and other choline esters) is approximately 730 to 1,040 mg/day (7 to 10 mmol/day).

Choline is available as a dietary supplement as choline chloride or choline bitartarte and as lecithin, which usually contains approximately 25 percent phosphatidylcholine or 3 to 4 percent choline by weight. There are no reliable estimates of the frequency of use or amount of these dietary supplements consumed by individuals in the United States and Canada. However, consumer exposure to manufactured choline chloride is likely to be insignificant compared to that occurring naturally in the diet, or produced *in vivo* by metabolic processes.

Adequate intake (AI) levels have been estimated in the USA by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. These were based on the amounts necessary to prevent liver abnormalities as indicated by serum enzyme levels. The AI for adult men is 550 mg/day of choline. The AI for adult women is 425 mg/day of choline, during pregnancy 450 mg/day and during lactation 550 mg/day, respectively (Standing Committee on the Scientific Evaluation of Dietary Reference Intake, 2000).

At an inadequate dietary intake in healthy men, decreased choline stores and liver damage (as assessed by elevated alanine aminotransferase) were reported (Zeisel *et al.*, 1991). Individuals fed with total parenteral nutrition solutions devoid of choline but adequate for methionine and folate developed fatty liver and liver damage; in some individuals, this resolved when a source of dietary

choline was provided (Buchman *et al.*, 1992; Buchman *et al.*, 1993; Buchman *et al.*, 1995; Burt, 1980; Chawla *et al.*, 1989; Tayek *et al.*, 1990; Shapira *et al.*, 1986; Sheard *et al.,* 1986).

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

Remark: The scientific literature of choline comprises thousands of published studies and reviews due to its role as a precursor for acetylcholine, phospholipids, and the methyl donor betaine and its use as dietary component and pharmaceutical.

Retrieval (at beginning of March 2003) by substance name (Choline) or CAS-No. (62-49-7) in MEDLINE and TOXLINE (the two most relevant medical /toxicological databases) resulted in 27575 and 395 hits, respectively. Retrieval for choline chloride (by name or CAS-No. 67-48-1) alone, however, resulted in zero hits in MEDLINE and 65 hits in TOXLINE suggesting that choline salts were not uncompromisingly encoded in these databases and therefore retrieval by choline chloride alone appeared not to be useful. Restricting the retrieval to the definite CAS-No. of choline (62-49-7) and using "human" as qualifier still resulted in 3029 hits in MEDLINE and TOXLINE.

Therefore with a focus on health and safety issues within the frame of the ICCA HPV programme, comprehensive reviews including those of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes of the Institute of Medicine (2000), Zeisel (2000) and Life Science Research Office (LSRO) / Federation of American Societies for Experimental Biology (FASEB) (1981) and studies cited in these reviews were chosen for this data set.

In some circumstances, available data for other choline salts have been evaluated to assist the weight of evidence approach for choline chloride. However, due consideration was given to potential toxicity exerted by the anion (e.g. salicylate).

3.1.1 Toxicokinetics, Metabolism and Distribution

No *in vitro* or *in vivo* animals studies available

Studies in Humans

In vivo Studies

Dietary choline is absorbed from the lumen of the small intestine. Pancreatic enzymes can liberate choline from dietary phosphatidylcholine (Politzer Shronts, 197). Before choline can be absorbed from the gut, some is metabolised by bacteria to form betaine and methylamines (Zeisel *et al*., 1983).

Fasting plasma choline concentrations vary from 9 to 20 µmol/L, with most subjects having plasma levels of 10 µmol/L (Savendahl *et al.*, 1997).

In a study with four patients receiving long-term total parenteral nutrition on 4 consecutive days and increasing doses, 7, 14, 28, and 56 mmol (8000 mg, highest dose) were intravenously infused over a 12-hour period in each subject. Plasma free choline at baseline before each application was 5.2 +/- 2.1 nmol/l. In all 4 subjects an overall increase in plasma choline level was observed during the 4 days of intermittent infusion; highest plasma levels were measured in most cases 6 h after start of the infusion and lowest levels after 12 h after the end of infusion period (Buchman *et al.*, 1994).

Conclusion

The data suggest a saturable elimination mechanism for choline that apparently operates only at large rates of administration. Tissue uptake and metabolism of choline may differ from normal in a choline-deficient state. The plasma free choline concentration would decline towards the resting level during the subsequent 12-hours period when infusion is interrupted.

3.1.2 Acute Toxicity

Studies in Animals

Inhalation

No valid data available.

Dermal

No valid data available.

Oral

Choline chloride is of very low acute oral toxicity. The oral LD_{50} in rats was determined to be between 3150 and ≥ 5000 mg/kg bw (corrected to 100% choline chloride) in two non-guideline studies (BASF AG 1963b, 1969a). Clinical symptoms after application were restlessness, increased frequency of respiration, hypoactivity, convulsions, ruffled coat, staggered gait and dyspnoea. Some animals had diarrhoea and at necropsy in the high dose groups in one study 3 out of 10 rats had inflamed lungs. In abstracts of literature, the reliability of which cannot be ascertained, the oral LD_{50} for rats was also in the range of 3400 to ≥ 6000 mg/kg bw (Merck 2000, NTP 2002, RTECS 2001, HSDB 2003).

The oral LD_{50} in mice was also in the range of 3900 to 6000 mg/kg bw in two studies of unknown reliability. There were no data available on clinical symptoms and histopathological findings (Henninghausen *et al.,* 1973, RTECS 2001).

Other Routes of Exposure

The LD_{50} for mice following intraperitoneal (i.p) administration of test substance was approximately 225 mg/kg bw calculated for the pure test substance. A 50% powder formulation, containing 29% of colloidal silicic acid and 21% of water was applied to 5 male and female mice in a dose range of 200 to 1600 mg/kg bw (referred to the 50% powder formulation). In the high dose range the mice died within 2 minutes (high dose) to 1 hour (640-800 mg/kg bw) after substance administration. Delayed mortality (1 day after application) was observed at 500 mg/kg bw (225 mg/kg referred to 100% active ingredient). Symptoms at ≥ 160 mg/kg bw (100% active ingredient) immediately after application were abdominal position, increased frequency of respiration, convulsions, dyspnoea, exophthalmus and cyanosis. At necropsy occasional adhesions in the area of the liver were observed (BASF AG 1969b).

In other study reports an LD_{50} i.p. of 320 mg/kg bw for mice (no further data; RTECS 2001) and 450 mg/kg bw for male rats have been reported (no further data; Sahu *et al.,* 1986, RTECS 2001). For symptoms and pathological findings no further data are available.

Studies in Humans

Mild hepatotoxicity was reported in patients receiving choline magnesium trisalicylate (1,500 mg twice daily for 8 days) (Cersosimo and Matthews, 1987) and one case of severe hypersensitivity

hepatitis after ingestion of choline magnesium trisalicylate (Nadkarni, *et al*., 1992). However, it is likely that hepatotoxicity was induced by salicylate rather than by choline.

Conclusion

Animal studies with choline chloride show a low acute toxicity after oral uptake (3,150 – ≥5000 mg/kg bw). No valid inhalation and dermal studies are available. No acute toxicity attributable to choline was observed in humans following oral doses of ≥3000 mg choline magnesium trisalicylate/day.

3.1.3 Irritation

Skin Irritation

Studies in Animals

In an old, non-guideline, non-GLP, study with occlusive exposure, only minor skin irritation (questionable reddening on the back of one rabbit) was found for a 70% aqueous formulation of the test substance. However, the degree of irritation would not be classifiable under GHS. The test substance was applied to the shaved skin on the back of two female White Viennese rabbits for 20 hours. Readings were performed at 24 hrs and 2, 5 and 8 days after application (BASF AG 1963c).

Irritation studies on the product formulation with approximately 30% content of "colloidal silicic acid" were discounted as no clear definition of the carrier material, and no independent test results were reported. Colloidal silicic acid, as well as colloidal silica, has to be regarded as mildly irritating to skin, eye and airways based on producer descriptions and literature though no test reports can be tracked down.

In a non-guideline study for which the reliability could not be ascertained, no signs of skin irritation were observed (BASF AG 1963a).

Studies in Humans

In a 21-Day Cumulative Irritation study on 25 subjects with self-perceived sensitive skin 0.5 % choline chloride aqueous solution, a soap bar containing 5 % choline chloride and a liquid body soap containing 5 % choline chloride was evaluated compared to controls. The analysis of the cumulative irritancy demonstrated no significant differences between the samples containing choline chloride and their respective choline chloride free controls (Colgate-Palmolive, 2003a).

Eye Irritation

Studies in Animals

In an old, non-GLP study, conducted broadly to OECD test guideline 405, only slight irritation was observed; however, the degree of irritation would not be classifiable under GHS. A 70% aqueous solution of the test substance was applied to one eye of one female and one male rabbit, the left eyes served as controls, to which saline was applied. After ten minutes, reddening of the eyes and tear secretion were observed. Slight reddening persisted up to three hours after application. No eye irritation or effects on the cornea were detectable after one day observation period. Post application readings were done after 1 and 3 hours, 1, and 8 days (BASF AG 1963c).

Irritation studies on the product formulation with approximately 30% content of "colloidal silicic acid" were discounted as no clear definition of the carrier material and no independent test results were reported. Colloidal silicic acid as well as colloidal silica have to be regarded as mildly

irritating to skin, eye and airways based on producer descriptions and literature though no definite test reports can be tracked down.

Conclusion

In rabbits, choline chloride may lead to a slight irritation of the skin and eye, which is, however, not sufficient to warrant a classification of choline chloride as an irritant under GHS.

3.1.4 Sensitisation

Studies in Animals

No data are available on sensitisation in animals.

Studies in Humans

In a Human Repeated Insult Patch Test on two hundred two subjects 0.5 % (w/v) choline chloride aqueous solution during the induction phase and 0.2 % (w/v) aqueous solution during the challenge phase was tested compared to controls. The results of the study showed no evidence of dermal sensitisation reactions elicited by choline chloride (Colgate-Palmolive, 2003b).

One case of acute contact dermatitis was reported in a woman who worked in a garden centre. Patch testing was positive at a concentration of 1% choline chloride in water and pet. Control tests using choline chloride in 10 patients were negative. (Fischer, 1984).

Conclusion

Only one case of contact allergy of choline chloride has been reported. The sensitisation potential of choline chloride is regarded as negligible.

3.1.5 Repeated Dose Toxicity

In animals, three repeat dose studies are available. One study which was by oral exposure in rats has been selected as key study because of its relevant route of exposure and a limited histopathological evaluation (Shivapurkar *et al.* 1986).

A second study that was conducted using intraperitoneal administration in rats has not been considered relevant to this assessment due to its route of exposure and partly insufficient documentation (Sahu *et al.,* 1986).

A third study conducted in guinea pigs by the intraperitoneal route of application has also been discarded due to choice of irrelevant route of exposure, shortcomings of documentation and limited histopathology (Sahu, 1989).

Studies in Animals

Oral

In a limited, non-OECD guideline, 72 weeks feeding study designed to investigate the impact of choline chloride on the liver tumour promoting activity of phenobarbital and DTT in DEN-initiated Fischer 344 rats, animals received approximately 500mg/kg bw/day of the test substance via feed. Further observations were made during a post-exposure period of 30 weeks, during which animals received the same untreated diet as the control group. Necropsy was performed at week 103. Histopathology was restricted to the liver and organs that developed gross abnormalities.

No significant differences between control groups and treated animals were observed with respect to survival rates, body weights, and relative liver weights. There was no increase in the number of neoplastic liver nodules, hepatocellular carcinomas, lung tumours, leukaemia or other tumours in choline-treated animals (Shivapurkar *et al.,* 1986). The NOAEL for choline chloride was ≥500mg/kg bw/day.

Studies in Humans

Oral

Fishy body odour, vomiting, salivation, sweating, and gastrointestinal effects were reported in patients with tardive dyskinesia and cerebellar ataxia treated with choline chloride at 150 and 220 mg/kg of body weight/day for 2 to 6 weeks (10 and 16 g/day, per oral, respectively) (Davis *et al*., (1975), Growdon *et al.* (1977), Lawrence *et al.* (1980)). The fishy body odour is believed to be due to the excretion of excessive amounts of trimethylamine, a metabolite produced by bacteriological action (Zeisel *et al*., 1991), and formation of methylamines from ingested choline and lecithin (Zeisel *et al*., 1983).

Oral administration of 10 g/day of choline chloride (which is equivalent to 7.5 g of choline), in a pilot study treating a small number of patients with Alzheimer's disease, resulted in a slight hypotensive effect (Boyd, *et al*. 1977), and this dose was regarded as a LOAEL by the Standing Committee on the Scientific Evaluation of Dietary Reference Intake, 2000.

Mild hepatotoxicity was reported in patients receiving choline magnesium trisalicylate (1,500 mg twice daily per oral for 8 days) (Cersosimo and Matthews, 1987) and severe hypersensitivity hepatitis after ingestion of choline magnesium trisalicylate in one case (Nadkarni *et al*., 1992), but it is likely that hepatotoxicity was induced by salicylate. Humans with and without cirrhosis have been treated with large doses of choline (6 g/day per oral for 4 weeks) with no resultant liver toxicity (Chawla*et al.*, 1989).

Tinnitus and pruritus have been reported in patients treated with doses of 3 g/day per oral of choline magnesium trisalicylate for 6 weeks. These side effects were transient and probably caused by salicylate (Mody *et al*., 1983).

In rare cases, oral ingestion of large amounts of choline up to 20 g/day for 3–4 weeks has been associated with depression (Davis *et al*., (1979), Tamminga *et al.*(1976)). Mild and transient Parkinsonian signs (bradykinesia, tremor, and rigidity) were observed at high doses (12.7 g/day per oral) of choline as a chloride in people with tardive dyskinesia (Gelenberg *et al*., 1979). In patients receiving up to 20 g of choline for the treatment of tardive dyskinesia and Huntington´s disease for four weeks no adverse effects were reported (Davis *et al*., 1978).

Conclusion

In a limited 72 weeks feeding study with Fischer 344 rats receiving approximately 500 mg/kg bw/day of the test substance via feed, no significant differences between control groups and treated animals were observed with respect to survival rates, body weights, and relative liver weights. Histological examination at autopsy was limited to the liver and organs showing gross abnormalities. No effects related to choline chloride intake were reported and ≥500 mg/kg bw/day was regarded as the NOAEL.

In humans, doses of 10 and 16 g/day administered for 2 to 6 weeks, were associated with fishy body odour, vomiting, salivation, sweating, and gastrointestinal effects in patients with tardive dyskinesia and cerebellar ataxia. Daily oral administration of 10g choline chloride (7.5g choline) had a slight hypotensive effect, but no other effects were noted. The Standing Committee on the Scientific Evaluation of Dietary Reference Intakes selected hypotension as the critical effect when

deriving a Tolerable Upper Intake Level, with fishy body odour as a secondary consideration. An uncertainty factor of 2 was incorporated because of the limited data regarding hypotension and the inter-individual variation in response to cholinergic effects. The value for the Tolerable Upper Intake Value for repeated exposure of adults was 3.5g/day choline.

3.1.6 Mutagenicity

The studies cited are comparable in quality to OECD guideline studies.

Studies in Animals

In vitro Studies

Negative results were obtained when choline chloride was investigated for its ability to induce gene mutations in bacteria. Three adequate, non-GLP Ames tests were conducted with *Salmonella typhimurium* strains TA 98, 100, 1535, 1537, 1538 up to 10,000 µg/plate and E. coli WP2 uvrA up to 5000 µg/ plate, with and without metabolic activation. No bacterial toxicity was observed in two of the studies; however, they were tested up to at least the limit dose of 5mg/plate. In the third study there was 50% survival at the top dose. The positive and negative controls gave appropriate results (Haworth *et al.* (1984), JETOC (1997), Litton Bionetics (1977), NTP (1983)).

NTP (1984) reported three experiments that investigated the potential of choline chloride to cause chromosomal aberrations. In the first experiment a small but statistically significant and dose related increase in simple aberrations was reported at 50 and 500 µg/ml in the absence of S9 only. No higher concentrations were examined. These results could not however be confirmed in two reliable studies using Chinese Hamster Ovary Cells in concentrations of choline chloride up to 5000 µg/ml Cytotoxicity (50% survival) started at 5000 µg/ml 100 cells were examined per dose and treatment group. Metabolic activation was with S9 from Aroclor induced rat livers (Bloom *et al.* (1982), Galloway *et al.* (1985), NTP (1984)).

Remark: The Galloway *et al*. (1985) study is cited in NTP as "Development of a standard protocol for *in vitro* cytogenetic testing with Chinese hamster ovary cells". In this publication a comparison of the results of two labs has been performed including choline chloride. So it is a key study cited by NTP. The Bloom *et al.* (1982) data have been available only as an abstract.

One sister chromatid exchange (SCE) assay gave an ambiguous result in two parallel studies (two different laboratories) in Chinese Hamster Ovary Cells performed at concentrations up to 500 µg/ml and 5000 µg/ml, respectively. Cytotoxicity (50% survival) was observed at 5000 µg/ml Metabolic activation was with S9 from Aroclor induced rat livers. Approximately 1000 chromosomes were examined per dose and treatment group. The increase (sporadic and not dose related) in SCEs that was observed with metabolic activation in laboratory two was not reproduced in laboratory one. Laboratory one showed a weak positive at the top dose without metabolic activation, but a comparison with laboratory two was not possible due to insufficient number of cells analysed. The results showed no concentration dependence (Bloom *et al.* (1982), Galloway *et al.* (1985), NTP (1984)).

In a third study with Chinese Hamster Ovary Cells concentrations up to 5000µg/ml (no data on cytotoxicity given), no increase in the number of sister chromatid exchanges was observed. Approximately 1000 chromosomes were examined per dose and treatment group (NTP, 1984).

In a gene conversion assay with *Saccharomyces cerevisiae* strain D4 choline chloride tested negative. The assay was performed in a dose range between 12.5 to 50 mg/ml with and without metabolic activation (Litton Bionetics, 1977).

In vivo Studies

No data are available.

Conclusion

Choline chloride does not have any structural alerts for genotoxicity. It did not produce gene mutations, clastogenicity or DNA damage when tested *in vitro*. It can be concluded from these studies that choline chloride does not have any mutagenic potential.

3.1.7 Carcinogenicity

No studies on carcinogenicity are available

3.1.8 Toxicity for Reproduction

Available studies on fertility and developmental toxicity are older studies and have not been conducted according to current test guidelines. However, generally they meet acceptable standards and are therefore adequate for this hazard assessment.

Studies in Animals

Effects on Fertility

In a study of unknown reliability due to limited reporting, male rats were exposed via intraperitoneal administration to 80mg/kg bw/day choline chloride for 12 and 24 days, respectively. Concurrent intake of choline chloride by feed was estimated to be 10-12 mg/kg bw/day. Compared to the concurrent control groups there were no differences in body weight gain or in the weights of testes, epididymides, liver, kidney and adrenals. At 2, 5, 8 and 12 days after the treatment period treated animals were sacrificed. Histopathological examination, including quantification of spermatogonia in zygotene and pachytene in ten randomly selected semniferous tubules at stage XII for each post exposure period was performed. After 12 days treatment, corresponding to one cycle of the semniferous epithelium, epithelial vacuoles, spermatogonia with pyknotic nuclei and cellular debris were noted 2 days after the end of the treatment. Five days later normal architecture of the seminiferous tubules was reported, attesting to a reversibility of the effects. Following the 24 days treatment damage at only a few tubules of stages I-IV were noted at day 2 post-treatment. Most tubules at stage IX-XIII were damaged. 5 and 8 days after treatment spermatogonia and spermatocytes appeared normal with some necrotic pachytene stages with an essential restoration to normal after 12 days (Vachhrajani *et al.*, 1993).

Although this study is of limited validity (due to the route of exposure and the limited reporting), and does not address female fertility or reproductive toxicity, there is no need for further testing to address the reproductive toxicity endpoint. There was an absence of gross abnormalities in the gonads in the limited repeated dose (accepting that there was no histological examination). Also, humans are exposed to significant amounts of choline chloride in the diet (1g per day), in addition to that produced metabolically in the body, as it is an essential component for a number of metabolic processes. Furthermore it has been widely used as an animal dietary supplement for decades with no reported adverse effects on fertility.

Developmental Toxicity

In a developmental toxicity study, mice were exposed, every other day, from gestation day 1 to 18 to choline chloride via feed over a dose range equivalent to 1250 to 20000 mg/kg bw/day. Maternal body weight gain was reduced in all but the lowest dose group. Determination of maternal weight

gain of dams with embryonic/fetal resorptions showed that, from 4160 mg/kg bw/day onwards, there was almost no net weight gain, and in the highest dose net weight loss. All fetuses were resorbed in the highest dose group. At 10800 and 4160 mg/kg bw/day 69% and 35%, respectively of embryonic/fetal lethality was recorded. No resorptions occurred in the low dose group. Developmental toxicity was observed in all but the lowest dose group, when maternal toxicity was apparent. No statistically significant increases in malformations were observed: 1.2% in the lowest dose group had cleft palate, compared to 1.02% in the historical controls. A low incidence of fused ribs was recorded (1 out of 166 fetuses in the low dose group, 1 out of 32 in the 10800 mg/kg bw/day group). This malformation was not assessed as dose related (BASF AG, 1966). NOAEL for maternal toxicity and developmental toxicity is 1250 mg/kg bw/day. A NOAEL for teratogenicity could not be determined because there were not sufficient pups.

It should be noted that the lowest dose used in this study was above the currently recommended top dose for non-toxic compounds, i.e. 1g/kg/day. The absence of any significant developmental toxicity effects at this level is reassuring, and supports the view that the compound does not have any significant developmental toxicity. The top dose used in this study was 20 times that recommended in the current OECD test guideline.

Studies in Humans

No data available.

Conclusion

Prolonged i.p. administration of choline chloride is toxic to the testes and causes damage to the seminiferous tubules. Under the testing protocol employed these lesions were reversible. However, this route of administration is not relevant for assessment of hazard to humans.

Developmental toxic effects have not been observed in the absence of maternal toxicity. Maternal and developmental toxicity started above the lowest dose which was already higher than the limit dose of 1000 mg/kg bw/day (NOAEL Maternal toxicity and developmental toxicity 1250 mg/kg bw/day). At the highest dose tested (20,000 mg/kg bw/day) 100% of the fetuses were resorbed.

3.2 Initial Assessment for Human Health

Animal studies with choline chloride show low acute toxicity following oral uptake (approximately 3150 – ≥5000 mg/kg bw). No acute toxicity attributable to choline was observed in humans following oral doses of ≥3000 mg choline magnesium trisalicylate/day.

In rabbits, choline chloride may lead to a slight irritation of the skin and eye, which is, however, not sufficient to warrant a classification of choline chloride as an irritant under GHS.

No data on sensitization in animals are available. The skin sensitisation potential of choline chloride for humans is regarded as negligible.

In a rat repeated dose study, using a single dose level of approximately 500 mg/kg bw/day given over 72 weeks via feed, with a post-observation period of 30 weeks, no significant effects were observed relative to controls with respect to survival rates, body weights and relative liver weights. Only limited pathological investigations were carried out at autopsy (gross examination with histological investigation of only the liver and any tissues showing gross abnormalities). No adverse effects were observed.

In humans, doses of 10 and 16 g choline chloride/day administered for 2 to 6 weeks, were associated with fishy body odour, vomiting, salivation, sweating. Gastrointestinal effects were reported in patients with tardive dyskinesia and cerebellar ataxia treated with choline chloride.

Repeated oral administration of 10g/day in patients with Alzheimer's disease produced a slight hypertensive effect, but no other adverse effects; this dose was regarded as a LOAEL;(it is equivalent to 7.5mg choline per day). The tolerable upper intake level applied for chronic daily use for adults was set at 3.5 g/day. Inadequate dietary intake decreases choline liver stores and may produce liver abnormalities as indicated by elevated serum alanine aminotransferase levels. As adequate intake for chronic daily use for adult men 550 mg/day of choline is recommended. The adequate intake for adult women is 425 mg/day of choline, during pregnancy 450 mg/day and during lactation 550 mg/day, respectively.

Choline chloride did not produce gene mutations, clastogenicity or DNA damage in *in vitro* mutagenicity studies; furthermore it has no structural alerts. Choline chloride does not have any genotoxic potential.

No developmental toxic effects were observed in mice after oral doses of 1250 mg/kg bw/day on gestation days 1 to 18. Higher doses, above the levels recommended currently and associated with maternal toxicity, did produce developmental toxic effects, but these were secondary to the maternal toxicity at the excessive doses used. The compound does not produce any significant developmental toxicity in the mouse.

Thus evidence from animal studies and from human exposure indicates that choline chloride has low toxicity, is not mutagenic and has no developmental toxicity. This is not unexpected in view of its presence in the diet and its production in metabolic processes in the body; it fulfils key roles in nerve transmission, cell membrane integrity, and lipid metabolism. Only limited animal data are available on effects on fertility, but the normal exposure of humans to appreciable amounts of choline chloride both from the diet and formed from normal metabolic processes, would argue against it having any significant adverse effects on fertility. This is supported by the fact that it has been widely used as an animal feed additive for decades with no apparent adverse effects being noted on fertility.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

The following acute and chronic toxicity tests with aquatic organisms are available (Table 2)

Table 2: Acute toxicity of choline chloride to aquatic organisms

Species	Method	Effect Concentration [mg/L]	Remark / Reference
Acute toxicity to fish			
Oryzias latipes **(freshwater species)**	**OECD 203 (flow-through system)**	**LC_{50} (96h) > 100 (nominal and measured)**	**purity of test substance: 100.2 %, reliability: 1; MOE Japan (1999a), KEY STUDY**
Leuciscus idus (freshwater species)	DIN 38412, part 15, static	LC_{50} (96h) > 10,000 (nominal)	no symptoms detectable; two tests with a) 78 % choline chloride watery solution (BASF AG, 1988b) and b) 50 % choline chloride as powder (BASF AG, 1988c) are available, non GLP, no analytics (reliability 2)
Limanda limanda (marine species)	according to OECD 203, semistatic	LC_{50} (96h) > 1,000 (nominal)	75 % choline chloride watery solution; limit test (only 1,000 mg/L tested); ICI (1983) (reliability 2)
Acute and chronic toxicity to aquatic invertebrates			
Daphnia magna **(freshwater species)**	**OECD 202 (static)**	**EC_{50} (48h) = 349 mg/l (nominal and measured)**	**purity of test substance: 100.2 %, reliability: 1; MOE Japan (1999b), KEY STUDY**
Daphnia magna (freshwater species)	Directive 79/831 EEC, C2, static	LC_{50} (48h) > 500 (nominal) NOEC (48hr) = 125	78 % choline chloride watery solution; non GLP, no analytics (reliability 2); BASF AG, 2003g
Daphnia magna **(freshwater species)**	**according OECD 211 (renewal system)**	**NOEC (21d) = 30.2 mg/L (nominal and measured)**	**purity of test substance: 100.2 %, reliability: 1; MOE Japan (1999c), KEY STUDY**
Acute toxicity to aquatic plants e.g. algae			
Pseudokirchneriella subcapitata **(freshwater species)**	**OECD 201**	**ErC_{50} (72h) > 1,000 (nominal and measured), 72h NOEC (growth rate) = 32**	**purity of test substance: 100.2 %, reliability: 1; MOE Japan (1999d), KEY STUDY**
Scenedesmus subspicatus (fresh water)	DIN 38412, part 9, static	$Er(b)C_{50}$ (72h) > 500(nominal), 72h NOEC (growth rate and biomass) >500	78 % choline chloride watery solution; non GLP, no analytics (reliability 2); BASF AG, 2003h

Toxicity to Microorganisms

The following toxicity tests with microorganisms is available (Table 3):

Table 3: Acute toxicity of choline chloride to microorganisms

Species	Method	Effect Concentration [mg/L]	Remark / Reference
Pseudomonas putida	DIN 38412, part 8, static	EC_{10} (17h) = 113 EC_{50} (17h) = 133 EC_{90} (17h) = 278 (each value referred to nominal values)	78 % choline chloride watery solution, reliability 2; BASF AG (2003i)

4.2 Terrestrial Effects

There are no data available concerning the toxicity to soil dwelling organisms, terrestrial plants or other non-mammalian terrestrial organisms.

4.3 Other Environmental Effects

There are no data available concerning other environmental effects.

4.4 Initial Assessment for the Environment

Choline chloride is a white crystalline solid but is marketed as an aqueous solution (70 – 75 % w/w in water) which is a colourless liquid with an amine-like odour. It is miscible with water in all proportions. It has a measured water solubility of ca. 650 g/L (calculated water solubility: 1,000,000 mg/L). Choline chloride is a quaternary amine salt, it dissociates in water into the corresponding positively charged quaternary hydroxyl alkylammonium ion and the negatively charged chloride ion. The calculated vapour pressure is $6.57*10^{-10}$ hPa at 25°C.The calculated Henry´s Law Constant is $2.06*10^{-11}$ Pa*m3/mole at 25°C. Due to the measured and calculated $\log K_{ow}$ of –3.77 and –5.15, respectively, and the calculated $\log K_{oc}$ of 0.37, bio- and geoaccumulation are not to be expected. Distribution modelling using Mackay Level I indicates water to be the main target compartment. Choline chloride was shown to be readily biodegradable according to OECD criteria in a MITI I-Test test 93 % BOD of ThOD within 14 days. A second test performed according to DIN 38409 part 43, found a $BOD_5/ThOD_5$ ratio of 75 %, confirming the ready biodegradability of choline chloride. In the atmosphere, the substance will be rapidly indirectly photodegraded by reaction with OH-radicals (calculated $t_{½}$ = 6.9 h) based on a 12h-day.

The following aquatic acute and chronic effect concentrations for freshwater as well as for marine species are available:

Fish

Oryzias latipes LC_{50} (96h) > 100 mg/l (nominal and measured)

Limanda limanda LC_{50} (96h) > 1,000 mg/l (nominal)

Invertebrates

Daphnia magna EC_{50} (48h) = 349 mg/l (nominal and measured)

Daphnia magna NOEC (21d) 30.2 mg/L (nominal and measured)

Crangon crangon EC_{50} (48h) > 1,000 mg/l (nominal)

Algae

Pseudokirchneriella subcapitata ErC_{50} (72h) > 1,000 mg/L (nominal and measured), 72h NOEC (growth rate) 32 mg/l.

Based on these data choline chloride is considered unlikely to be harmful to aquatic organisms.

Based on the available data, choline chloride is expected to exert toxicity by a non-specific mode of action. Based on the most sensitive acute toxicity data for *D. magna* with an EC_{50} of = 349 mg/L, a $PNEC_{aqua}$ of 3.49 mg/l can be derived by applying an assessment factor of 100, according to the Technical Guidance Document for the EU risk assessment procedure. Since there are NOECs available for Daphnia and algae and it is unlikely that the fish long-term NOEC would be lower than these values, derivation of $PNEC_{aqua}$ by applying an assessment factor of 10 to the Daphnia

NOEC of 30.2 mg/l could also be considered, resulting in a $PNEC_{aqua}$ of 3.02 mg/l, which is close to the other value obtained using the acute data.

5 RECOMMENDATIONS

Human Health:

The chemical is currently of low priority for further work due to its low hazard profile.

Environment:

The chemical is currently of low priority for further work due to its low hazard profile.

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ANNEX

Details of the literature search used

The data banks searched are indicated below.

The scientific literature of choline comprises thousands of published studies and reviews due its functions as a precursor for acetylcholine, phospholipids, and the methyl donor betaine and its use as dietary component and pharmaceutical.

Retrieval (at beginning of March 2003) by substance name (Choline) or CAS-No. (62-49-7) in MEDLINE and TOXLINE (the two most relevant medical /toxicological databases) resulted in already 27575 hits, respectively 395 hits. Retrieval for choline chloride (by name or CAS-No. 67-48-1) alone, however, resulted in zero hits in MEDLINE and 65 hits in TOXLINE suggesting that choline salts were not uncompromisingly encoded in these databases and therefore retrieval by choline chloride alone seems to be not useful. Restricting the retrieval to the definite CAS-No. of choline (62-49-7) and using "human" as qualifier still resulted in 3029 in MEDLINE and TOXLINE.

Therefore with a focus on health and safety issues in the frame of ICCA HPV program comprehensive reviews including those of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes of the Institute of Medicine (2000), Zeisel (2000) and Life Science Research Office (LSRO) / Federation of American Societies for Experimental Biology (FASEB) (1981) and studies cited in these reviews were chosen for this data set.

Toxicology

Date of last literature search: March 2003

JETOC

RTECS

AGRICOLA

CABA

CANCERLIT

TOXCENTER

TOXLINE

JICST-EPLUS

LIFESCI

TOXLIT

EMBASE

ESBIOBASE

EMBAL

HEALSAFE

CSNB

MEDLINE

RIFM-FEMA database

IRIS

ATSDR TOX. PROFILES

atsdr TOX: FAQS

chemfinder

civs

gestis

ginc

nicnas

ntp

Ecology

Date of last literature search: 04 Nov 2002

AQUASCI

BIOSIS

EMBASE

ESBIOBASE.

LIFESCI

OCEAN

POLLUAB

SCISEARCH

TOXCENTER

TOXLINE

ULIDAT

datalog

chemfate

biodeg

aquire

HSDB

I U C L I D

D a t a S e t

Existing Chemical ID: 67-48-1
CAS No. 67-48-1
EINECS Name choline chloride
EC No. 200-655-4
Molecular Weight 139.63 g/mol
Molecular Formula C5 H14 N O .Cl

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

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

Memo: master

Printing date: 28-FEB-2005
Revision date:
Date of last Update: **28-FEB-2005**

Number of Pages: **127**

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

1.0.1 Applicant and Company Information

Type: lead organisation
Name: Akzo Nobel Chemicals NV
Street: Stationsplein 4
Town: 3800 AE Amersfoort
Phone: +31 33 467 6420
Telefax: +31 33 467 6171
Email: Chris.Braun@AkzoNobel-Chemicals.com

Flag: Critical study for SIDS endpoint
22-JUL-2004

Type: cooperating company
Name: AirProducts Chemicals
Country: Netherlands

Flag: Critical study for SIDS endpoint
22-JUL-2004

Type: cooperating company
Name: BASF AG
Country: Germany

Flag: Critical study for SIDS endpoint
22-JUL-2004

Type: cooperating company
Name: Taminco NV
Country: Belgium

Flag: Critical study for SIDS endpoint
22-JUL-2004

1.0.2 Location of Production Site, Importer or Formulator

1.0.3 Identity of Recipients

1.0.4 Details on Category/Template

1.1.0 Substance Identification

Mol. Formula: C5 H14 Cl N O
Mol. Weight: 139.63 g/mol

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004

1.1.1 General Substance Information

Substance type: organic
Physical status: solid
Colour: colourless
Odour: faint amine-like
Flag: non confidential, Critical study for SIDS endpoint

14-JAN-2004 (1)

Physical status: liquid
Purity: >= 75 - % w/w
Colour: clear, aqueous
Odour: nearly odourless

Test substance: Choline chloride solution 75% (aqueous solution)
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (2)

Remark: Colour: max. 50 Hazen
Flag: non confidential, Critical study for SIDS endpoint
25-MAY-2004 (3)

1.1.2 Spectra

1.2 Synonyms and Tradenames

(.beta.-Hydroxyethyl)trimethylammonium chloride

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

(2-Hydroxyethyl)trimethylammonium chloride

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

(b-Hydroxyethyl)trimethylammonium chloride

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Bilineurin chloride

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Biocolina

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Biocoline

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Cholinchlorid

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

Choline chloride

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

Choline, chloride

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Choline, chloride (8CI)

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

Cholinium chloride

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

Ethanaminium, 2-hydroxy-N,N,N-trimethyl-, chloride

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Ethanaminium, 2-hydroxy-N,N,N-trimethyl-, chloride (9CI)

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

Hepacholine

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Hormocline

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Lipotril

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Luridin chloride

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Neocolina

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Paresan

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Trimethyl(2-hydroxyethyl)ammonium chloride
Flag: non confidential, Critical study for SIDS endpoint

02-DEC-1992

1.3 Impurities

EINECS-Name: organic impurities (trimethylamine + glycol + chloroethanol)
Contents: <= .15 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
25-MAY-2004 (3)

CAS-No: 75-50-3
EC-No: 200-875-0
EINECS-Name: trimethylamine
Mol. Formula: C3 H9 N
Contents: <= .05 - % w/w

Test substance: Choline chloride solution 75% (aqueous solution)
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (2)

CAS-No: 107-21-1
EC-No: 203-473-3
EINECS-Name: ethane-1,2-diol
Mol. Formula: C2 H6 O2
Contents: <= .05 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
25-MAY-2004 (3)

EINECS-Name: heavy metals as lead
Contents: <= .002 - % w/w

Flag: non confidential, Critical study for SIDS endpoint
25-MAY-2004 (3)

1.4 Additives

CAS-No: 7732-18-5
EC-No: 231-791-2
EINECS-Name: water
Mol. Formula: H2 O
Contents: ca. 25 - % w/w
Funct. of add.: Solvent

Test substance: Choline chloride solution 75% (aqueous solution)
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (2)

1.5 Total Quantity

Quantity: ca. 85000 tonnes produced in 1984

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (5)

1.6.1 Labelling

Labelling: no labelling required (no data available)

Remark: no classification available in the EU for this substance
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (6)

1.6.2 Classification

Classified: no classification required (no data available)

Remark: no classification available in the EU for this substance
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (6)

1.6.3 Packaging

1.7 Use Pattern

Type: use
Category: Food/foodstuff additives

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (1)

Type: use
Category: Food/foodstuff additives

Remark: Selected food additive classified as a nutrient and dietary supplement. [R5]
Source: R5: Doull, J., C.D. Klaassen, and M. D. Amdur (eds.). Casarett and Doull's Toxicology. 2nd ed. New York: Macmillan Publishing Co., 1980. 558
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (7)

Type: use
Category: Food/foodstuff additives

Remark: Choline chloride is very important in the animal feedstuff industry [51].
Source: [51] J. Gropp: Chemie und Ernährung - BASF Forum Tierernährung am 28./29. 10. 1982, Verlag Wissenschaft und Politik, p. 111.
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (5)
Type: use
Category: Pharmaceuticals

Remark: liver protection substance
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (1)

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

Orig. of Subst.: Synthesis
Type: Production

Remark: reaction of trimethylamine and concentrated hydrochloric acid, followed by treatment with ethylene oxide under pressure [R1]
Source: R1: SRI
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (7)

Orig. of Subst.: Synthesis
Type: Production

Remark: Industrially, N-alkylated ethanolamines are produced almost exclusively by batchwise or continuous reaction of primary, secondary, or tertiary amines with ethylene oxide [44][45][46].

Trialkylammonium chlorides yield the corresponding 2-hydroxyethylammonium chlorides (e.g. cholin chloride):

(H3C)3NH+ Cl- + (CH2)2O -> (CH3)3N+-CH2CH2-OH Cl-

Choline chloride can also be prepared from trimethylamine and ethylene chlorohydrin, but this route has no commercial significance.
Source: [44] Hori Todashi: "Alkylamines and Derivatives V - Ethoxylated Chemicals," Bosei Kanri 20 (1976) no. 12, 37.
[45] IG-Farbenindustrie, DE 650 574, 1928.
[46] Carbides and Carbon Chem. Corp., US 1 904 013, 1927 (E. Reid, D. C. Lewis).
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (5)

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

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

Country: Germany
Remark: ID-Number: 1134
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (8)

1.8.4 Major Accident Hazards

1.8.5 Air Pollution

1.8.6 Listings e.g. Chemical Inventories

Type: EINECS
Additional Info: EINECS No. 200-655-4

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Type: ENCS
Additional Info: ENCS No. 2-341X
ENCS No. 9-1994X

Remark: For ENCS chemical class or category name, refer to ENCS No. 2-341
For ENCS chemical class or category name, refer to ENCS No. 9-1994
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Type: ECL
Additional Info: ECL Serial No. KE-20909

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Type: other: SWISS
Additional Info: SWISS No. G-8733

Remark: SWISS CLASSIFICATION:
Giftliste 1 (List of Toxic Substances 1), 31 May 1999.
Toxic Category 5.
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Type: TSCA

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Type: DSL

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Type: AICS

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Type: PICCS

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Type: other: ASIA-PAC

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (4)

Type: other: FDA/CFSAN Everything Added to Food in the United States (EAFUS) Database, 5/16/03
Additional Info: EAFUS document number: 1925

Country: North America
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (9)

Type: Annex I, Council Regulation (EEC) No. 793/93

Country: Western Europe
Source: EU. Annex I to Council Regulation 793/93 on the evaluation and control of the risks of existing substances: List of existing substances produced or imported within the Community in quantities exceeding 1000 tonnes/year. O.J. (L 84) 1, 5 Apr 1993.
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (9)

Type: other: EU. Cosmetics Directive 76/768/EEC, Annex II - Prohibited Substances
Additional Info: as amended through by 2003 OJ (L 238) 23, 25 September 2003

Country: Western Europe
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (9)

Type: other: OECD. Representative List of High Production Volume Chemicals (HPV)

Country: Western Europe
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (9)

Type: other: Switzerland. BAG Giftliste 1 (Stoffe), April 2002 [Toxics List 1 (Substances)]
Additional Info: as amended by 2003 BBl., number 42, page 7058, 28 October 2003

Swiss Identification Number: G-8733
Toxicity Category: 5

Country: Switzerland
Remark: Toxicity Category 5 is determined by acute oral lethal doses of 2000 - 5000 mg/kg in small animals; however, other factors may be taken into consideration regarding data in other types of animals or other affects whether subacute, subchronic or chronic.
Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (9)

1.9.1 Degradation/Transformation Products

Type: degradation product

CAS-No: 7664-41-7
EC-No: 231-635-3
EINECS-Name: ammonia, anhydrous

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (6)

Type: degradation product
CAS-No: 7647-01-0
EC-No: 231-595-7
EINECS-Name: hydrogen chloride

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (6)

Type: degradation product
EINECS-Name: nitrogen oxides

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (6)

Type: degradation product
CAS-No: 630-08-0
EC-No: 211-128-3
EINECS-Name: carbon monoxide

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (6)

Type: degradation product
CAS-No: 124-38-9
EC-No: 204-696-9
EINECS-Name: carbon dioxide

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (6)

1.9.2 Components

1.10 Source of Exposure

1.11 Additional Remarks

Memo: Hazardous reactions:
strong exothermic reaction/heat development with:
- strong bases
- humidity
- strong oxidizing agents
- strong acids

Flag: non confidential, Critical study for SIDS endpoint
14-JAN-2004 (6)

1.12 Last Literature Search

Type of Search: Internal and External
Chapters covered: 5.10
Date of Search: 06-NOV-2002

26-JUN-2003

Type of Search: Internal and External
Chapters covered: 5.10
Date of Search: 12-MAR-2003

26-JUN-2003

1.13 Reviews

2.1 Melting Point

Decomposition: yes at = 303 - 305 degree C

GLP: no data
Test substance: other TS: choline chloride, no further data

Remark: Products of decomposition: NH3, HCl, NOx, CO, CO2
Reliability: (4) not assignable
Secondary literature
17-JUN-2004 (10)

Decomposition: yes at = 180 degree C

Method: other
GLP: no data
Test substance: other TS: choline chloride, colourless solid substance, purity >=98%

Reliability: (4) not assignable
Manufacturer/producer data without proof
16-JUN-2004 (11)

Decomposition: at = 247 degree C

Method: other: method unknown
GLP: no data
Test substance: other TS: choline chloride, no data on purity are available

Reliability: (2) valid with restrictions
secondary literature, but reliable peer-reviewed source of data
Flag: Critical study for SIDS endpoint
19-JUL-2004 (12)

Decomposition: yes at > 100 degree C

Method: other
GLP: no data
Test substance: other TS: 70+-1% choline chloride, 30% water, less than 0.05% impurities

Remark: At a temperature of > 100 °C the water evaporate and decomposition of the salt starts.
No information about method and year the study was conducted are avilable.
Reliability: (4) not assignable
Manufactorer/producer data without proof
17-JUN-2004 (13)

2.2 Boiling Point

2.3 Density

Type: density
Value: = 1.1 g/cm³ at 20 degree C

Method: other
GLP: no data
Test substance: other TS: choline chloride solution, 75 %

Test substance: As the value indicated here refers to a solution the density of the pure substance may differ.
Reliability: (4) not assignable
Manufacturer/producer data without proof
16-JUN-2004 (14)

Type: density
Value: = 1.1 g/cm³ at 20 degree C

Test substance: other TS: 70 +-1% choline chloride, 30% water, less than 0.05% impurities

Remark: As the values indicated here refer to a solution the density of the pure substance may differ.
Result: The following values are given:

Temperatue [°C]	Density [g/ml]
-20	1.12
0	1.11
20	1.10

Reliability: (4) not assignable
Manufacturer/producer data without proof
Flag: Critical study for SIDS endpoint
13-JUN-2003 (13)

Type: bulk density
Value: = 430 kg/m3

Test substance: other TS: choline chloride, colourless solid substance, purity >=98%

Reliability: (4) not assignable
Manufacturer/producer data without proof
13-JUN-2003 (11)

Type: bulk density
Value: = 400 - 600 kg/m3

GLP: no data
Test substance: other TS: Choline chloride powder, 60 %

Reliability: (4) not assignable
Manufacturer/producer data without proof
16-JUN-2004 (15)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = 8 hPa at 20 degree C
Decomposition: no

Method: other (measured): static
GLP: no data
Test substance: other TS: 70+-1% choline chloride, 30% water, less than 0.05% impurities

Remark: Information about the year and the type of study performed is not available. Further, the values presented here are most likely to refer to the water effected by the high concentration of salt. Measured data on vapour pressure for the pure choline chloride are not available.

Result:

Temperature in °C	Vapour pressure in hPa
30	14.5
40	25
50	41
60	66
70	100
80	157
90	230
100	340

Reliability: (4) not assignable
Manufacturer/producer data without proof
17-MAY-2004 (13)

Value: = 10 hPa at 20 degree C

GLP: no data
Test substance: other TS: Choline chloride solution, 75 %

Remark: Information about the year and the type of study performed is not available. Further, the values presented here are most likely to refer to the water effected by the high concentration of salt. Measured data on vapour pressure for the pure choline chloride are not available.
Result: At 100°C a vapour pressure of 413 hPa is given.
Reliability: (4) not assignable
Manufacturer/producer data without proof
17-JUN-2004 (16)

Value: = .000000000657 hPa at 25 degree C

Method: other (calculated): using MPBPWIN v1.40 (Modified Grain Method)
Year: 2003

Remark: This value refers to the pure choline chloride.
The originally calculated value by the program was 4.93*10-10 mmHg. After converting this value using the equation: 1 mmHg = 133.322 Pa, a value of 6.57*10-10 hPa could be derived.

The input parameter of the program are:
melting point: 305 °C
boiling point: 380.89°C
Reliability: (2) valid with restrictions
Scientifically acceptable calculation
Flag: Critical study for SIDS endpoint
28-JUN-2004 (17)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = -5.16 at 25 degree C

Method: other (calculated): via SRC KOWWIN v1.66
Year: 2003

Remark: The values are calculated using the SRC KOWWIN v1.66 method which is Meylan & Howard (1995).
For choline chloride a value of -5.1554 was estimated.
Reliability: (2) valid with restrictions
Scientifically acceptable calculation
18-MAY-2004 (18)

Partition Coeff.: octanol-water
log Pow: = -3.77 at 25 degree C

Method: other (measured): test procedure according to an internal BASF standard, comparable to OECD 107
Year: 1988
GLP: no

Result: Results of the 3 determinations:
1. trial: Pow = 1.8 mg/l octanol / 11.83 g/l water = 0.00015
2. trial: Pow = 2.7 mg/l octanol / 16.32 g/l water = 0.00016
3. trial: Pow = 5 mg/l octanol / 25.82 g/l water = 0.00019

Mean: Pow 0.00017; log Pow -3.77
Test condition: Test vessels were prepared containing accurately measured amounts of the test substance (three trials: 0.2939 g, 0.4203 g, or 0.6474 g) together with 25.0 ml octanol-1 and 25 ml aqua dest.
After achieving equilibrium the aqueous phase was separated and the concentration of the test substance in water and in octanol was determined by ion-pair chromatography on a NPIC-NS1 column (effluent 0.002 mol/l hexanesulfonic acid with 1.0% [V/V] acetonitril). Triplicate determinations were performed.
Test substance: Choline chloride solution, 75 % in water
Reliability: (2) valid with restrictions
Study meets generally accepted scientific principles
Flag: Critical study for SIDS endpoint
17-JUN-2004 (19)

2.6.1 Solubility in different media

Solubility in: Water
Value: ca. 650 g/l
pH value: = 6 - 7
Conc.: 10 g/l degree C

Test substance: other TS: choline chloride powder 50%: choline chloride 50+-1 %, silicic acid ca. 35 % (colloidal), water ca. 15%

Remark: No data about the method used and temperature at which the study was performed are available.
Reliability: (4) not assignable

Manufacturer/producer data without proof
Flag: Critical study for SIDS endpoint
13-JUN-2003 (13)

Solubility in: Water

Method: other: no data
GLP: no data

Remark: Choline chloride at different amounts and different specifications was tested; pH values are also indicated. The values corresponds to the amount of test substance added to water.

Choline chloride in water [g/L]	pH-value (measured, at 20°C)
10	6 - 7 (Choline chloride, 60 % powder)
50	5 - 6 (Choline chloride, 75 % solution)
100	4 - 6 (Choline chloride, 98 %)

No information about the method which was used and the year the study was conducted are available.
Reliability: (4) not assignable
Manufactorer/producer data without proof
17-JUN-2004 (15) (16) (11)

Solubility in: other: water and ethanol
Descr.: other: freely soluble in both media

Method: other: no data
GLP: no data

Reliability: (4) not assignable
Manufactorer/producer data without proof
17-JUN-2004 (11)

Solubility in: Water

Method: other: no data
GLP: no data
Test substance: other TS: Choline chloride, most likely pure

Result: Choline chloride is indicated to be very soluble in water.
Test substance: no data on purity are available
Reliability: (2) valid with restrictions
secondary literature, but reliable peer-reviewed source of data
17-JUN-2004 (12) (20)

Solubility in: Water

Method: other: calculated via WSKOW v1.40
Year: 2003

Remark: The value refers to the pure choline chloride.
Result: The water solubility is 1,000,000 mg/L at 25°C.
Reliability: (2) valid with restrictions
scientifically acceptable method

29-JUN-2004 (21)

2.6.2 Surface Tension

2.7 Flash Point

2.8 Auto Flammability

Value: = 330 degree C

Method: other: according to VDI 2263 chapter 2.6 (BAM-oven)
GLP: no
Test substance: other TS: choline chloride powder 50%: choline chloride 50%, silicic acid 35 % (colloidal), water 15%

Method: The BAM-oven is a 170 mm long electrically heated pipe-oven which is horizontally arranged. The dust sample is blown with air from the face of the oven axially against the impact plate. The test is performed on the sample fraction having a particle size less than 63 µm. The oven is heated up to a maximum temperature of 600°C. Ignition is considered to have taken place when the dust blown into the oven ignites or decomposes producing flames or explosion and this means that the flap at the end of the BAM-oven has to be lifted and flames become visible.
Remark: The ignition temperature of airborne dust on a hot surface was determined.
Reliability: (2) valid with restrictions
Meets national standard methods with acceptable restrictions.
Flag: Critical study for SIDS endpoint
17-JUN-2004 (22)

2.9 Flammability

Result: other: This chemical is relatively nonflammable

Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature; no further data available
13-JUN-2003 (23)

17-JUN-2004

2.10 Explosive Properties

Result: not explosive
Test substance: other TS: Choline chloride 50x, waterfree

Remark: The substance is not considered an explosive substance

because the exothermic decompostion energy, determined by a DTA (Differential Thermal Analysis), is less than 500 J/g. No plot was created from the DTA.
Test condition: Heating of 61.5 mg test substance; temperature 30-400°C, speed of heating: 2°C per min.
Reliability: (2) valid with restrictions
Expert judgement
Flag: Critical study for SIDS endpoint
17-JUN-2004 (24) (25)

Result: other: the dust of this product has explosive properties

Method: other: according to VDI 2263 chapter 2.1.1 "Hartmannrohr"
GLP: no
Test substance: other TS: choline chloride powder 50%: choline chloride 50%, silicic acid 35 % (colloidal), water 15%

Method: In a standardised test apparatus with a contents of 20 litre, a small number of tests is performed over a wide range of concentrations (normally from 30 g/m3 to 2000 g/m3) to determine whether or not the dust is explosible. Production of a whirled up dust/air-mixture was carried out at room temperature and a pressure of 1 bar (abs). The ignition took place by a spark. The particle size was 3 < d < 330 µm.
Remark: Minimal ignition energy is > 1300 mJ.
Reliability: (2) valid with restrictions
Meets national standard methods, acceptable for assessment
Flag: Critical study for SIDS endpoint
13-JUN-2003 (26)

Result: other: the dust of this product has explosive properties

Method: other: no data
GLP: no data
Test substance: other TS: Choline chloride powder, 60 %

Reliability: (4) not assignable
Manufacturer/producer data without proof
17-JUN-2004 (15)

2.11 Oxidizing Properties

Result: no oxidizing properties

Remark: Choline chloride is not considered an oxidizing substance because the compound contains chlorine which is bonded only to hydrogen.
Reliability: (4) not assignable
Expert judgement
Flag: Critical study for SIDS endpoint
17-JUN-2004 (25)

2.12 Dissociation Constant

Method: other: derivation

Remark: Choline chloride is a quaternary amine salt, it dissociates in water into the corresponding positively charged quaternary hydroxyl alkylammonium ion and the negatively charged chloride ion. Data using different amounts of choline chloride show that the lowest pH value of 4 was determined at a water solubility of 100 mg/L (see below). Therefore, choline chloride can be considered a weak acid.

Amount of choline chloride in water [g/L]	pH-value (measured, at 20°C)
10	6 - 7
50	5 - 6
100	4 - 6

Reliability: (2) valid with restrictions
scientifically acceptable and comprehensible
17-MAY-2004 (15) (16) (11)

2.13 Viscosity

Test type: other
Value: = 21 mPa s (dynamic) at 20 degree C

Method: other: according to DIN 51757
GLP: no
Test substance: other TS: 70+-1% choline chloride, 30% water, less than 0.05% impurities

Remark: No information about the year the study was conducted is available.
Result: Viscosity at 0°C = 50 mPa x s
at -20°C = 149 mPa x s
Reliability: (4) not assignable
Manufacturer/producer data without proof
Flag: Critical study for SIDS endpoint
13-JUN-2003 (13)

2.14 Additional Remarks

Memo: odour: amine-like

Test substance: choline chloride, colourless solid substance, purity >=98%
Reliability: (4) not assignable
Manufacturer/producer data without proof
13-JUN-2003 (11)

Memo: odour: slightly amine-like

Test substance: choline chloride powder 50%: choline chloride 50+-1 %, silicic acid ca. 35 % (colloidal), water ca. 15%
Reliability: (4) not assignable
Manufacturer/producer data without proof
13-JUN-2003 (13)

Memo: The test substance is hygroscopic

Test substance: choline chloride, colourless solid substance, purity >=98%
Reliability: (4) not assignable
Manufacturer/producer data without proof
17-MAY-2004 (11)

Memo: odour: nearly odourless

Test substance: 70+-1% choline chloride, 30% water, less than 0.05% impurities
Reliability: (4) not assignable
Manufacturer/producer data without proof
13-JUN-2003 (13)

Memo: other

Remark: white powder, hygroscopic
bulk density: 0.5-0.7 g/ml
stability: practically unlimited storage at 20-30°C
Test substance: choline chloride powder 50%: choline chloride 50+-1 %, silicic acid ca. 35 % (colloidal), water ca. 15%
Reliability: (4) not assignable
13-JUN-2003 (13)

Memo: other

Remark: clear, aqueous solution;
stability: practically unlimited storage;
heat capacity: 0.578 kcal/kg °C
refraction index nD = 1.4500-1.4600.
Test substance: 70+-1% choline chloride, 30% water, less than 0.05% impurities
13-JUN-2003 (13)

Memo: Dangerous product of decomposition: HCl

Reliability: (4) not assignable
Manufacturer/producer data without proof
13-JUN-2003 (15)

Memo: combustibility

Method: The combustibilty was measured according to VDI 2263 chapter 1.2.
The principle of the test is based on the investigation whether and at which degree seasoned dust inflammated by firing may spread out.
The test is performed on the sample fraction having a particle size less than 250 µm. The test material is poured onto a fire-resistant plate to form an unbroken strip of product. An electrically heated, glowing platinum wire at a temperature of approx. 1000°C is dipped into the test substance at one end of the product strip for approx. 5 seconds.
Result: The study was carried out at room temperature and at 100°C. At room temperature the flammability was comparable to D(+)-lactose (score 3 out of 6 scores), whereas at 100°C the flammability was comparable to sulphur (score 5).

The test substance is not highly combustible at room

temperature.
Test substance: Choline chloride powder 50%: choline chloride 50%, silicic acid 35 % (colloidal), water 15%
Reliability: (2) valid with restrictions
Meets national standard methods with acceptable restrictions.
17-JUN-2004 (27)

Memo: crystallization temperature

Remark: The crystallization temperature is indicated with - 24°C. No details on the test substance used and no data on GLP are available.
Reliability: (4) not assignable
Manufactorer/producer data without proof
16-JUN-2004 (16)

Memo: solidifying or pour point

Remark: At a temperature of - 64°C the solution thickened. No data on GLP are given.
Test substance: 70+-1% choline chloride, 30% water, less than 0.05% impurities
Reliability: (4) not assignable
Manufactorer/producer data without proof
16-JUN-2004 (13)

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1500000 molecule/cm³
Rate constant: = .000000000018639 cm³/(molecule * sec)
Degradation: = 50 % after 6.9 hour(s)

Method: other (calculated): via SRC AOP v1.90
Year: 2004

Remark: The calculation is based on a 12h-day.
Reliability: (2) valid with restrictions
Scientifically acceptable calculation
29-JUN-2004 (28)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Rate constant: = .0000000000186393 cm³/(molecule * sec)
Degradation: = 50 % after 20.7 hour(s)

Method: other (calculated): AOP v1.90
Test substance: other TS: choline chloride, no further data

Remark: The calculation is based on a 24h-day.
Reliability: (2) valid with restrictions
Scientifically acceptable calculation
29-JUN-2004 (29)

3.1.2 Stability in Water

Remark: Choline chloride is a quaternary ammonium salt and dissociates in water. No measured data on the stability of choline chloride in water are available.
Reliability: (2) valid with restrictions
scientifically accepted rule of dissociating chemicals
11-NOV-2004

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

Type of measurement: other
Medium: air

Remark: Emission during production in the year 2000 less than 5 kg per year.
German Emission Register 2000.
Declaration of the BASF AG.
Reliability: (2) valid with restrictions
Expert judgement
Flag: Critical study for SIDS endpoint
29-OCT-2003 (30)

Type of measurement: other: choline in food

Remark: Choline is found in egg yolk, vegetable and animal fat, mostly as lecithin.
Reliability: (4) not assignable
Secondary literature
29-OCT-2003 (31)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Type: adsorption
Media: water - soil
Method: other: calculated via SRC PCKOCWIN v1.66
Year: 2003

Result: logKoc = 0.37 (Koc = 2.34)
Reliability: (2) valid with restrictions
Scientifically acceptable calculation
Flag: Critical study for SIDS endpoint
29-OCT-2003 (32)

Type: volatility
Media: water - air
Method: other: calculated via SRC HENRYWIN v3.10
Year: 2003

Result: Henry's Law Constant = 2.06*10E-11 Pa*m³/mole (at 25°C; bond method)
Reliability: (2) valid with restrictions
Scientifically acceptable calculation
Flag: Critical study for SIDS endpoint
29-OCT-2003 (33)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water
Method: other (calculation): Mackay Level I V2.11
Year: 2003

Remark: The following input paratemeter were used for the calculation:
molecular mass: 139.63 g/mol
water solubility: 100000 g/m3 (calculated)
vapour pressure: 6.00E-08 Pa
log Kow: -5.155
data temperature: 25°C
melting point: 274°C
The Henry's Law Constant calculated by the program itself is 8.38*E-11 Pa*m³/mole.

Input parameter for the program:

	Volume (m³)	Density (kg/m³)	org. C (g/g)	fish lipid (g/g)
Air	6.0E+09	1.185		
Water	7.0E+06	1000		

Soil	45000	1500	0.02	
Sediment	21000	1300	0.05	
susp. Sed.	35	1500	0.167	
Fish	7	1000		0.05
Aerosole	0.012	1500		

Result: Based on this calculation the pure choline chloride will be mainly distributed into the compartment water (100 %).
Only very small amounts are distributed into the other compartments: air: 2.90*E-09 %
soil: 5.53*E-08 %
sediment: 5.60*E-08 %
supended sediment: 3.59*E-10 %
fish: 3.50*E-11 %
aerosol: 1.95*E-08 %

Reliability: (2) valid with restrictions
Scientifically acceptable method

Flag: Critical study for SIDS endpoint

24-SEP-2004 (34)

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge
Concentration: 100 mg/l related to Test substance
Degradation: = 93.5 % after 14 day(s)
Result: readily biodegradable
Deg. product: not measured

Method: OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year: 1974
GLP: no
Test substance: other TS: choline chloride, no data on purity were provided

Remark: No data concerning the kinetic of the biodegradation of choline chloride are available. Nevertheless, Tunkel et al. (2000) published that if in a 14 day MITI-I test a substance will be degraded up to >= 60 % the substance can be stated as readily biodegradable.

Test condition: The sludge was collected at 10 different sampling sites of Japan and mixed:
- industrial sludge (1x)
- municipal sludge (3x)
- surface water (3x river samples)
- surface soil (3x soil samples)

Then fresh and old activated sludge were mixed:
- 5 L of the filtrate of a supernatant of an activated sludge in present use and 500 mL of the filtrate of newly collected sludge
- cultivation at pH 7.0 +/- 1.0 and aeration

Culture:
- after 30 min supernatant corresponding to about 1/3 of the whole volume of the sludge mixture was removed
- adding dechlorinated water of equal volume and aeration

- addition of 0.1 (w/v)% synthetic sewage (consisting of a solution of glucose, peptone and monopotassium phosphate)
Concentration of activated sludge: 30 mg/l
Reference substance: aniline
Preparation of test solutions (300 mL vessels):
- 1 vessel: water + test substance
- 1 vessel: sludge and test substance
- 1 vessel: sludge and aniline
- 1 vessel: control blank
Conditions of cultivation:
-25 +/- 1°C for 14 days
Validity criterium:
- percentage biodegradation of aniline (by BOD) were beyond 40 % and 60 % after 7 days and 14 days, respectively

Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
19-JUL-2004 (35) (36)

Type: aerobic
Inoculum: other: see test condition
Result: other: readily biodegradable (but not according to OECD criteria)

Method: other: see freetext
GLP: no
Test substance: other TS: choline chloride 78%, no further data

Method: According to national guidelines called:
Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Kurzzeitverfahren (H43). DIN 38409 Part 43, 1982
Result: BOD5 = 1280 mg/g test substance
ThOD = 1710 mg/g test substance
BOD5/ThOD = 75%
Test condition: Measurement of the biochemical oxygen demand according to the dilution method BOD5 in relation to the theoretical oxygen demand.
Inoculum: effluent of an industrial sewage plant (BASF AG).
Reliability: (2) valid with restrictions
Meets national standard methods with acceptable restrictions, details of the study confined to the above
Flag: Critical study for SIDS endpoint
29-OCT-2003 (37) (38)

Type: aerobic
Inoculum: activated sludge

Method: other: comparable to OECD Guide-line 301 C
GLP: no
Test substance: other TS: choline chloride, no further data

Remark: The publication is an overview on 300 substances examined on biodegradability in Japan.
Result: Well-biodegradable substance (no further data).
Test condition: - 30 ppm active sludge and 100 ppm test substance
- test temperature 25+-2°C; pH 7.0 +- 0.1
- test period 2 weeks
- positive control: aniline
- measurement of biodegradation: percentage calculated from

oxygen consumption

Criteria:
If the percentage biodegradation from the oxygen consumption exceeds 30 % after two weeks from the beginning of the test and the result of a direct analysis is at least this value the TS is judged as well-biodegradable.

Reliability: (4) not assignable
Documentation insufficient for assessment; Restrictions: data confined to the above

28-OCT-2003 (39)

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

Species: other: fish
BCF: = .59

Method: other: calculated according to Veith et al. (1979) as cited in the TGD (2003)

Result: Using the equation according to the TGD (2003) and the measured logKow of -3.77 a BCF for the fish of 0.59 can be calculated.

Equation:
log BCF (fish) = 0.85 + logKow - 0.70

Reliability: (2) valid with restrictions
Scientifically and generelly accepted calculation; based on a measured logKow

Flag: Critical study for SIDS endpoint

29-OCT-2003 (40)

Species: other: fish
BCF: = 3.16

Method: other: calculated via SRC BCFWIN v2.14
Year: 2003

Reliability: (2) valid with restrictions
Scientifically acceptable calculation

Flag: Critical study for SIDS endpoint

29-OCT-2003 (41)

3.8 Additional Remarks

Memo: No monitoring data of neither choline chloride nor choline (CAS 62-49-7) from the effluent of the BASF sewage plant are available.

Reliability: (2) valid with restrictions
Expert Judgement

28-OCT-2003 (42)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 10000
LC50: > 10000
LC100: > 10000

Method: other: according to DIN 38412 Part 15
Year: 1982
GLP: no
Test substance: other TS: choline chloride, 78% of active ingredient, presumably dissolved in water

Remark: Cholin chloride as 78 % active ingredient dissolved in water was tested. No correction to the account for the presence of water has been made.
Result: Results:
- no mortality in any group
- LC50 (96h) > 10,000 mg/L (1 % significance level)
- No animals showed adverse effects in negative control and in the treatments
- Positive control conducted with chloroacetamide, LC50 (48h) = 32 mg/l (normal sensitivity)

Test condition: Closely followed the German Industrial Standard Guideline Number DIN 38 412, Part 15 (June 1982) using a static exposure procedure.
The Golden Orfe (L. idus), golden variety, was used.
Aeraton: slight
Duration of housing and adaptation: about 6 months (water temperature 11-20°C)
Duration of adaptation to test conditions: 3 days
Withdrawal of food before exposure: 1 day before and during exposure
Light/dark cycle: 16h/8h
Body length: 7.8 cm (range: 6.6-9.1)
Body weight: 4.7 g (range: 3.1-6.7)
Loading: 4.7 g fish/l test water
Test design: 10 fish were used per concentration and an untreated control, at nominal concentrations of 0, 5,000 and 10,000 mg/l.

measured pH values:

concentration	pH				
(nominal, mg/l)	1h	24 h	48 h	72 h	96 h
5,000	7.7	7.6	7.7	7.7	7.7
10,000	7.8	7.8	7.9	7.9	7.9
control	7.6	7.3	7.8	7.7	7.7

measured oxygen concentrations

concentration (nominal, mg/l):	O2				
	1h	24 h	48 h	72 h	96 h
5,000	7.7	7.5	7.8	7.6	7.8
10,000	8.0	8.2	8.4	8.4	8.4

control 7.4 7.5 7.9 7.7 8.0

The concentrations used were chosen based on a range finding study.
The test substance was added to the test water without any prior treatment. Subsequently, the fish were added to the water.
Test vessel: All-glass aquarium non-sealed (30 x 22 x 24 cm)
Dilution water chemistry: reconstituted freshwater was prepared from demineralized tap water that was resalted by the addition of 294.0 mg/l CaCl2*2H2O, 123.3 mg/l MgSO4*7H2O, 63.0 mg/l NaHCO3 and 5.5 mg/l KCl. The test water had a total hardness of 2.5 mmol/l, an acid capacity of 0.8 mmol/l and a pH about 8.0. The water temperature was 21°C. As control test water without test substance was used.

Reliability: (2) valid with restrictions
Test procedure in accordance with national guideline and comparable to OECD guideline 203 with acceptable restrictions; no analytical monitoring, no GLP study
10-NOV-2004 (43)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 10000
LC50: > 10000
LC100: > 10000

Method: other: according to DIN 38412 Part 15
Year: 1982
GLP: no
Test substance: other TS: choline chloride, 50% powder

Result: Results:
- no mortality in any group
- LC50 (96h) > 10,000 mg/L (1 % significance level)
- No animals showed adverse effects in negative control and in the treatments
- Positive control conducted with chloroacetamide, LC50 (48h) = 31 mg/l (normal sensitivity)

Test condition: Closely followed the German Industrial Standard Guideline Number DIN 38 412, Part 15 (June 1982) using a static exposure procedure.
The Golden Orfe (L. idus), golden variety, was used.
Aeraton: slight
Duration of housing and adaptation: about 6 months (water temperature 11-20°C)
Duration of adaptation: 3 days
Withdrawal of food before exposure: 1 day before and during exposure
Body length: 6.2 cm (range: 5.7-6.9)
Body weight: 2.7 g (range: 2.2-3.4)
Loading: 2.7 g fish/l test water
Test design: 10 fish were used per concentration and an untreated control, at nominal concentrations of 0, 5,000 and 10,000 mg/l.

measured pH values:
concentration pH

(nominal, mg/l)	1h	24 h	48 h	72 h	96 h
5,000	7.7	7.8	7.8	7.8	7.9
10,000	7.5	7.6	7.7	7.7	7.7
control	8.0	7.9	7.9	8.0	8.0

measured oxygen concentrations

concentration (nominal, mg/l):	O2				
	1h	24 h	48 h	72 h	96 h
5,000	7.9	8.3	8.4	8.8	8.7
10,000	8.2	8.4	8.7	8.9	9.0
control	7.7	7.4	7.7	8.5	8.4

The concentrations used were chosen based on a range finding study.
The test substance was added to the test water without any prior treatment. Subsequently, the fish were added to the water.
Test vessel: All-glass aquarium non-sealed (30 x 22 x 24 cm)
Dilution water chemistry: reconstituted freshwater was prepared from demineralized tap water that was resalted by the addition of 294.0 mg/l $CaCl_2*2H_2O$, 123.3 mg/l $MgSO_4*7H_2O$, 63.0 mg/l $NaHCO_3$ and 5.5 mg/l KCl. The test water had a total hardness of 2.5 mmol/l, an acid capacity of 0.8 mmol/l and a pH about 8.0. The water temperature was 21+/1°C.
As control test water without test substance was used.

Reliability: (2) valid with restrictions
Test procedure in accordance with national guideline and comparable to OECD guideline 203 with acceptable restrictions; no analytical monitoring, no GLP study

29-OCT-2003 (44)

Type: semistatic
Species: Limanda limanda (Fish, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: > 1000

Method: other: see freetext
Year: 1983
GLP: no data
Test substance: other TS: choline chloride, 75% aqueous solution

Result: All animals in the treatment group survived; 1 animal in the control group was found dead after 72 h.
Test condition: Test system:
- 10 animals per concentration were tested
- average weight of animals: 5.36 g
- as test solution Brixham seawater (15 L per concentration) was used and changed every 24 hours changed
- animals exposed for 96 h to 0 and 1000 mg test substance per l test medium
- the nominal test temperature was 15°C.

Reliability: (2) valid with restrictions
Meets generally accepted standards, acceptable for assessment, no guideline study, no GLP, one dose, data confined to the above.
Flag: Critical study for SIDS endpoint

18-MAY-2004 (45)

Type: static
Species: other: Ptychocheilus oregonensis, Oncorhynchus kisutch, Oncorhynchus tshawytscha
Limit Test: yes

Method: other: see freetext
GLP: no
Test substance: other TS: choline chloride, no further data

Result: 10 ppm of the test substance had no observable adverse effects on behavior or mortality of P. oregonensis, O. kisutch, and O. tshawytscha.
Test condition: Details:
- fish length 5-10 cm (all species)
- acclimatization (fish starved) 3-24 h
- study temperature 51 °F (10.5 °C)and pH 7.2
- hardness: 0-17 ppm
- only 1 animal of each species tested in the same vessel (4 l)
- loading 5g fish/L
Reliability: (3) invalid
Unsuitable test system
29-OCT-2003 (46)

Type: flow through
Species: Oryzias latipes (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC0: >= 100
LC50: > 100
Limit Test: yes

Method: OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year: 1999
GLP: yes
Test substance: other TS: Wako Pure Chemical Industries, Ltd., Lot. No.;PAR1681, Purity = 100.2%

Method: - Test Organisms:
a) Supplier: Test organisms were obtained from private reproduction in Japan.
b) Size (length and weight): 2.1cm (1.8 - 2.3cm) in length; 0.17 g (0.13 - 0.22 g) in weight.
c) Age: Not described.
d) Any pretreatment: Test organisms were acclimated for more than 12 days before testing. During acclimination, test fishes were fed with TETRAMIN fish food. The mortality of the test organisms for 7 days before testing was below 5%. LC50(96 hr) for a reference substance (copper sulfate pentahydrate) was 0.18 mg/L.

-Test substance: choline chloride
a) Empirical Formula: C5H14NO.Cl
b) Molecular Weight: 139.63 g/mol
c) Purity: = 100.2 %
d) Water Solubility: High

-Test Conditions:
a) Dilution Water Source: Dilution water was prepared from dechlorinated industrial water (drinkable water grade). This

water was aerated after residual chlorine removal by activated carbon treatment.
b) Dilution Water Chemistry: pH : 7.8; Total hardness (as CaCO3) : 30 mg/L
c) Exposure Vessel Type: 3 L glass beaker
d) Nominal Concentrations: control and 100 mg/L (limit test)
e) Vehicle/Solvent and Concentrations: Not used.
f) Stock Solutions Preparations and Stability: Test substance was diluted with dilution water. Test substance was stored in freezer. The stability of the chemical was confirmed by IR absorption spectrum. Under the stock condition, IR spectrum of the test substance at the end of test was same at the start.

g) Number of Replicate: 1
h) Fish per Replicates: 10
i) Renewal Rate of Test Water: 5 times per a day
j) Water Temperature: 24+/1C
k) Light Condition: 16:8 hours, light-darkness cycle
l) Feeding: None
m) Aeration : None

-Analytical Procedure:
The test concentrations were measured at the start, 48th and 96th hours using HPLC.

-Statistical Method:
a) Data Analysis: None
b) Method of calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Arithmetic mean (show below), however the nominal concentration was used for calculation.

Result:

- Measured Concentrations:
The test concentrations were measured at the start of the test, 48h and 96h hours using HPLC

Nominal Conc. [mg/l]	Measured Concentration (mg/L)				
0 Hour	48 Hour	96 Hour	Mean	% of Nominal	
Control	---	---	---	---	---
100	106	101	96.0	101	101

Water chemistry (pH and DO and temperature in test): Water chemistry and temperature were measured for each concentration everyday:
pH: 7.3 - 7.7
DO: 6.4 - 8.2 mg/L
Water Temperature: 23.7 - 23.9C

-Effect Data (mortality):
LC50 (96hr) > 100 mg/L (nc)
LC0 (96hr) > 100 mg/L (nc)
The LC50 value and its 95% confidence limits could not be determined because the test was conducted as a limit test.

- Cumulative Mortality:
None of test organisms were killed during exposure period at both control and 100 mg/l.

Measured Conc. [mg/l]	Cumulative Number of Dead (% Mortality) 24 h	48 h	72 h	96h
Control	0 (0)	0 (0)	0 (0)	0 (0) 100
	0 (0)	0 (0)	0 (0)	0 (0)

Other Effect: Symptoms of toxicity was not observed during test period.

- Calculation of toxicity values: The calculation of toxicity values was the nominal concentration.

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint

28-FEB-2005 (47)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC0: = 125
EC50: > 500
EC100: > 500

Method: other: according to Directive 79/831 EEC, C2
Year: 1984
GLP: no
Test substance: other TS: choline chloride, purity 78%; presumably 78% solution in water

Remark: Choline chloride as 78 % active ingredient dissolved in water was tested. No correction to the account for the presence of water has been made.

Result: Results:

concentration in mg/l	number of mobile daphnids after 0 h	3 h	6 h	24 h	48 h
0	20	20	20	20	20
31	20	20	20	20	20
62	20	20	20	20	20
125	20	20	20	20	20
250	20	20	20	19	16
500	20	20	20	14	11

- exposure time 3h: EC0 = 500 mg/l, EC50 > 500 mg/l
- exposure time 6h: EC0 = 500 mg/l, EC50 > 500 mg/l
- exposure time 24h: EC0 = 250 mg/l, EC50 > 500 mg/l
- exposure time 48h: EC0 = 125 mg/l, EC50 > 500 mg/l

Results in the control:
- valid negative control (immobility 0% after 48 h)

Test condition: Stock solution and test solution and their preparation:
- stock solution 500 mg test substance/L

- stock solution diluted to the below mentioned concentrations with test water
- prepared nominal concentrations: 0 (negative control), 31, 62, 125, 250, 500 mg/l (no positive control).

Test system:
- Test animals: Daphnia magna Straus
- Test volume per animal: 2 mL
- Test volume: 10 mL
- Number of replicates (individuals/vessel): 4 (5)
- Test temperature: 292.0-294.0 °K
- Light/dark cycle: 16h/8h
- test water specifications: pH = 8.0; total hardness 3.00 mmol/L; Ks up to pH 4.3 = 0.75 mmol/L
- Dissolved oxygen: 8.99-9.40 mg/l (start of exposure); after 48 h: 8.54, 6.7, 6.32, 3.68, 3.5, 2.64 mg/l at 0, 31, 62, 125, 250, 500 mg/l test substance, respectively
- pH at start of exposure: 7.97-8.06 (in control pH 7.94); pH after 48 h exposure: 7.11-7.64 (in control pH 8.0)

Monitoring of test substance:
- Test performed without concentration control analysis.

statistics
- No data

Reliability: (2) valid with restrictions
Guideline study with acceptable restrictions, no GLP study, no analytical monitoring

29-JUN-2004 (48)

Type: semistatic
Species: Crangon crangon (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50 : > 1000

Method: other: Semi-static test
Year: 1983
GLP: no data
Test substance: other TS: choline chloride, 75% aqueous solution

Result:

Concentration (mg/L)	% Survivers 3h	24h	48h	72h	96h
1000	100	95	90	80	80
560	100	85	80	75	75
0	/*	85	80	80	75

The 96h LC50 was calculated to be > 1000 mg/L.

*probably a typing error; after 24h 85 % survivers were observed, so after 3 h it must have been 100 % and not zero.

Test condition: A semi-static test protocol was used with 20 animals/test and the test solutions were changed every 24 hours. The average weight of the shrimps was 1.14 g and Brixham sea-water (10 litres/test concentration) was used throughout. The nominal test temperature was 15 degree C.

Reliability: (4) not assignable
Test report not longer available
18-MAY-2004 (49)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** yes
EC0: = 180
EC50: = 349
EC100: = 1000
Limit Test: no

Method: OECD Guide-line 202
Year: 1999
GLP: yes
Test substance: other TS: Wako Pure Chemical Industries, Ltd., Lot. No.;PAR1681, Purity = 100.2%

Method: -Test Organisms:
a) Age: < 24 hours old
b) Supplier/Source: Test organisms were obtained from the National Institute of Environmental Studies (Japan).
c) Any pretreatment: Parental daphnids were acclimated for 2 - 4 weeks on test condition before testing. During acclimatization, test daphnids were fed with Chlorella vulgaris, 0.15 mg carbon/day/individual. Juveniles in batches of high mortality and contain resting eggs and males were not used as test indivisuals. EC50 (48hr, immobility) for reference substance (potassium dichromate) was 0.079 mg/L.

-Test substance: choline chloride
a) Empirical Formula: C5H14NO.Cl
b) Molecular Weight: 139.63g/mol
c) Purity: = 100.2 %
d) Water Solubility: High

-Test Conditions:
a) Dilution Water Source: Dilution water was prepared from dechlorinated industrial water (drinkable water grade). This water was aerated after residual chlorine removal by activated carbone treatment.
b) Dilution Water Chemistry: pH : 7.8; Total hardness (as CaCO3): 27 mg/L
c) Exposure Vessel Type: 100 mL test solution in a 100 mL glass beaker.
d) Nominal Concentrations: control, 100, 180, 320, 560 and 1000 mg/L
e) Vehicle/Solvent and Concentrations: Not used.
f) Stock Solutions Preparations and Stability: Test substance was diluted with dilution water. Test substance was stored in freezer. The stability of the chemical was confirmed by IR absorption spectrum. Under the stock condition, IR spectrum of the test substance at the end of test was same at the start.
g) Number of Replicates: 4
h) Individuals per Replicates: 5
i) Water Temperature: 20+/-1°C
j) Light Condition: 16:8 hours, light-darkness cycle
k) Feeding: None
l) Aeration : None

- Analytical Procedure: Test concentrations were measured at the start and the end of test using HPLC.

- Statistical Method:
a) Data Analysis: EiC50 and 95% confidence intervals were calculated by Probit method.
b) Method of Calculating Mean Measured Concentrations: The nominal concentration was used for calculation.

Result: - Measured Concentrations:
The test concentrations were measured at the start and the end during test period.

Nominal Conc. [mg/l]	Measured Conc. [mg/L]		% of Nominal Conc.	
	0 Hour	48 Hour	0 Hour	48 Hour
Control	N.D.	N.D.	---	---
100	104	104	104	104
180	176	174	98	97
320	337	346	105	108
560	562	580	100	104
1000	1050	1040	105	104

Water chemistry (pH, DO and temperature in test):
Water chemistry and temperature were measured for control and each concentration at the start and the end of test: pH: 7.8 - 8.0
DO: 8.7 - 8.9 mg/L
Water Temperature: 19.9 - 20.4°C

-Effect Data:
EC50 (48hr) = 349 mg/L (nc) (95% C.I.: 303 - 401 mg/L)
EC100 (48hr) = 1000 mg/L (nc)
NOEC (48hr) = 180 mg/L (nc)

-Mortality or Immobility:
None of test organisms were immobilized the behavior at control. The lowest concentration from which the test organisms were immobilized was 320 mg/L at 48 h.

Cumulative Number of Immobilized Daphnia (% Immobility)

Nominal Conc. [mg/l]	24 h	48 h
Control	0 (0)	0 (0)
100	0 (0)	0 (0)
180	0 (0)	0 (0)
320	1 (5)	8 (40)
560	6 (30)	9 (95)
1000	17 (85)	20 (100)

-Calculation of toxic values: Nominal concentration

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint

28-FEB-2005 (50)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Pseudokirchneriella subcapitata
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: = 32
EC50: > 1000
Limit Test: no

Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 1999
GLP: yes
Test substance: other TS: Wako Pure Chemical Industries, Ltd., Lot. No.;PAR1681, Purity = 100.2%

Method: -Test Organisms:
a) Supplier/Source: Obtained from subculture in Kureha Special Laboratory Co., Ltd.
b) Method of Cultivation: Sterile
c) Strain Number:ATCC22662
d) Any pretreatment: Acclimated for 4 days before testing

-Test substance: choline chloride
a) Empirical Formula:C5H14NO.Cl
b) Molecular Weight: 139.63g/mol
c) Purity: = 100.2 %
d) Water Solubility: High

- Test Conditions:
a) Medium: OECD medium
b) Exposure Vessel Type: 100 mL medium in a 300mL glass Erlenmeyer flask
c) Nominal Concentrations: control, 1.0, 3.2, 10, 32, 100, 320 and 1000 mg/L
d) Vehicle/Solvent and Concentrations: Not used
e) Sock Solution Preparations and Stability: Test substance was diluted with OECD medium. Test substance was stored in freezer. The stability of the chemical was confirmed by IR absorption spectrum. Under the stock condition, IR spectrum of the test substance at the end of test was same at the start.

f) Number of Replicates: 3
g) Initial Cell Number: 10,000 cells/mL
h) Water Temperature: 23+/-2C
i) Light Condition: 4000 - 5000 lux, continuously j) Shaking: 100 rpm
---
- Analytical Procedure: Test concentrations were measured at the start and the end of test using by HPLC after removing algal cells by a centrifuge.

- Statistical Method:
a) Data Analysis: Probit method for EC50 if applicable. 1-way ANOVA (a=0.05) and Dunnett's method (a=0.05, both side) for NOEC, after Bartlett's homoscedastic test.

b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): The nominal concentration was used for calculation.

Result: - Measured Concentrations :
Test concentrations were measured at the start and the end of test using by HPLC. All of them, the deviation from the nominals were less than +/- 10%.

Nominal Conc. [mg/l]	Measured Conc. [mg/L]		% of Nominal Conc.	
	0 h	72 h	0 h	72 h
Control	N.D.	N.D.	---	---
1.0	0.8	1.0	80	100
3.2	3.0	3.1	94	97
10	10.0	10.1	100	101
32	31.3	32.1	98	100
100	103	108	103	108
320	328	345	103	108
1000	1070	1070	107	107

-Water chemistry (pH and temperature in test): pH was measured for control and each concentration at the start and the end of test. At the start and the end of test, the pH was 8.4 - 8.6 and 9.2 - 10.4, respectively. Temperature in algal culture cabinet was maintained 23.0C during test period.
pH: 8.4 - 10.4
temperature: 23 +/-2°C

-Effect Data: Rate Method
EC50 (0-72h) : Cannot calculated.
NOEC (0 - 72 hr) = 32 mg/L. (nc)

- Growth Inhibition (%) of Pseudokirchneriella subcapitata

Measured Conc. [mg/l]	Growth rate, Inhibition and Cell density		
Rate (Average) u(0-72hr)	Inhibition(%) Im(0-72hr)	Cell density(72hr) mg/L	
Control	13.3	---	839200
1.0	13.2	0.480	821700
3.2	13.2	0.821	809200
10	13.1	1.08	800000
32	13.1	1.67	779200
100	12.8	3.99 **	703300
320	12.6	4.84 **	677500
1000	12.5	6.21 **	638300

Growth Curves:
Exponential growth phase was kept during 72 hours.

- Calculation of toxic value: Nominal concentration
** Indicates a significant difference (a=0.01) from the

control.
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
28-FEB-2005 (51)

Species: other algae: Scenedesmus subspicatus CHODAT SAG 86.81 (new name: Desmodesmus subspicatus)
Endpoint: other: growth rate and biomass
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: > 500
EC10: > 500
EC50: > 500

Method: other: according to DIN 38412 Part 9
GLP: no
Test substance: other TS: choline chloride, 78% solution in water

Result: Control:
- valid negative control (610x10E3 cells per ml medium after 96 h)
- valid results in uninoculated samples

Inhibtion of growth rates after 72 h:

	Concentration in g/l					
	10	25	50	100	250	500
Inhibition in % of control	0.8	0.5	2.0	2.6	1.3	1.8

ErC10 (72h) > 500 mg/l
ErC50 (72h) > 500 mg/l

Inhibtion of algal biomass after 72 h:

	Concentration in g/l					
	10	25	50	100	250	500
Inhibition in % of control	0.0	-6.3	-3.8	-9.4	5.6	-4.4

EbC10 (72h) > 500 mg/l
EbC50 (72h) > 500 mg/l

Results after 96 h exposure:
- Similar effects on growth rate and biomass as after 72 h exposure; in the high dose group slight decrease in growth rate (inhibition 3.5% of control), biomass (inhibition 6.1% of control), and cell density (531x10E3 per ml medium, control see above)
- No effect on photosynthesis detected (measured at termination of experiments)

Test condition: Test system:
- nominal concentrations: 0, 10, 25, 50, 100, 250, 500 g/l; test substance added to the test water without solvent
- pH 8.03-8.27 in all groups at the start of exposure and pH 6.58 (high concentration) up to 8.32 (control) measured at the end of exposure period
- temperature 21.5°C in all groups during exposure period
- in vivo chlorophyll fluorescence measured in 4 samples per concentration after 0, 24, 48, 72, 96 h; fluorescence also determined without addition of algae (2 samples per

concentration)
- positive control: potassium dichromate (results not presented)
- statistical calculations according to: Tallarida & Jacob (1979) The dose-response relation in pharmacology, Springer, 98-103

Reliability: (2) valid with restrictions
Meets national standard methods with acceptable restrictions, no GLP, no analytical monitoring, details confined to the above

08-OCT-2004 (52)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: other bacteria: Pseudomonas putida DSM 50026
Exposure period: 17 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC10: = 113
EC50: = 133
EC90 : = 278

Method: other: DIN 38412 Teil 8, draft; inhibition of cell multiplication
Year: 1986
GLP: no
Test substance: other TS: choline chloride, 78% solution in water

Result: Control:
- valid negative control;
- valid results in uninoculated samples.

Inhibition after 17 h:

	Nominal concentration in mg/l					
	50	100	150	200	250	300
E at 436 nm in % of control value	105	116	15.2	16.3	15.0	6.1

Test condition: Performance of the test:
- medium: according to DIN 38412, part 8
- stock solution of the test substance: 1250 mg/l
- temperature: 293°K
- test substance concentrations tested: 0, 50, 100, 150, 200, 250, 300 mg/l
- 4 inoculated samples and 1 uninoculated sample per concentration were measured

Reliability: (2) valid with restrictions
Meets national standard methods with acceptable restrictions, no GLP, no analytical, monitoring, details confined to the above

Flag: Critical study for SIDS endpoint
29-JUN-2004 (53)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Endpoint: reproduction rate
Exposure period: 21 day(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: = 30.2
LOEC: = 95.5
EC50: = 58.9

Method: OECD Guide-line 211
Year: 1999
GLP: yes
Test substance: other TS: Wako Pure Chemical Industries, Ltd., Lot. No.;PAR1681, Purity = 100.2%

Method: -Test Organisms:
a) Age: < 24 hours old
b) Supplier/Source: Test organisms were obtained from the National Institute of Environmental Studies (Japan).
c) Any pretreatment: Parental daphnids were acclimated for 20 days on test condition before testing. During acclimatization, test daphnids were fed with Chlorella vulgaris, 0.15 mg carbon/day/individual. Mothers of test individuals were selected from batches which were not observed death individuals and any resting-eggs and male daphnids. EC50 (48hr, immobility) for reference substance (potassium dichromate) was 0.079 mg/L.

-Test substance: choline chloride
a) Empirical Formula: C5H14NO.Cl
b) Molecular Weight: 139.63g/mol
c) Purity: = 100.2 %
d) Water Solubility: High

-Test Conditions:
a) Dilution Water Source: Dilution water was prepared from dechlorinated industrial water (drinkable water grade). This water was aerated after residual chlorine removal by activated carbone treatment.
b) Dilution Water Chemistry: pH : 7.3; Total hardness (as CaCO3): 37 mg/L
c) Exposure Vessel Type: 80 mL test solution in a 200 mL glass Erlenmeyer flask.
d) Nominal Concentrations: control, 3.2, 10, 32 and 100 mg/L
e) Vehicle/Solvent and Concentrations: Not used.
f) Stock Solutions Preparations and Stability: Test substance was diluted with dilution water. Test substance was stored in freezer. The stability of the chemical was confirmed by IR absorption spectrum. Under the stock condition, IR spectrum of the test substance at the end of test was same at the start.

g) Number of Replicates: 10
h) Individuals per Replicates: 1

i) Water Temperature: 20+/-1C
j) Light Condition: 16:8 hours, light-darkness cycle
k) Feeding: 0.15 - 0.18 mg carbon/day/individual (Chlorella vulgaris: Green Algae)
l) Aeration : None

- Analytical Procedure: The test concentrations were measured six times during test period for both renewal and old test solution using HPLC.

- Statistical Method:
a) Data Analysis: LC50 and EC50: LC50 and their 95%c.l. cannot be calculated. EC50 and 95% C.I. were calculated by probit method. NOEC and LOEC: The cumulative number of juveniles produced per adult in control and test concentration after 21 days was tested by Dunnett multiple comparison procedure.
b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Time-weighted Mean

Result:

- Effect: reproduction

- Measured concentrations: The test concentrations were measured for both renewal and old test solution at the start of the test and after 1st, 10th, 11th, 20th and 21st day.

Nominal Conc. [mg/l]	Measured Concentration [mg/l]					
Date	0	1	10	11	20	21
	New	Old	New	Old	New	Old
Control	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3.2	3.1	3.1	3.3	2.5	3.2	1.9
10	9.7	9.7	10.3	8.1	9.9	6.6
32	33.5	32.8	34.2	25.7	32.1	23.7
100	104	101	104	87.0	101	77.3

---

new: freshly prepared test solution.
old: test solutions 24 hours after freshly prepared.

Nominal Conc. [mg/l]	Percent of Nominal Concentration (%)					
Date	0	1	10	11	20	21
	New	Old	New	Old	New	Old
Control	---	---	---	---	---	---
3.2	97	97	103	78	100	59
10	97	97	103	81	99	66
32	105	103	107	80	100	74
100	104	101	104	87	101	77

---

new: freshly prepared test solution.
old: test solutions 24 hours after freshly prepared.

- Water chemistry (pH, DO and and temperature in test): Water chemistry and temperature were measured for control and each concentration at the start of test and after 1st, 10th, 11th,

20th and 21st days.
pH: 7.0 - 7.7
DO: 6.7 - 8.1 mg/L
Water Temperature: 20.3 - 21.0°C
Total hardness (as CaCO3): 23 - 37 mg/L

-Effect Data (Reproduction):
LC50 (21days) > 95.5 mg/L (parental mortality) (mc)
EC50 (21days) = 58.9 mg/L (mc; 95%C.I.: 37.7 - 83.7 mg/L)
NOEC (21days) = 30.2 mg/L (mc)
LOEC (21days) = 95.5 mg/L (mc)
mc: based on Time-weighted mean of measured concentrations

- Cumulative Number of Died Parental Daphnia: Mortality rate of parental Daphnia in the control was 0%.

Nominal Conc. [mg/l]	Cumulative Number of Died Parental Daphnia After 21 days (Mortality rate, in %)
Control	0 (0)
3.2	0 (0)
10	2 (20)
32	0 (0)
100	0 (0)

-Time (days) to First Brood Production

Nominal Conc. [mg/l]	Time (days) to First Brood Production Mean
Control	8.3
3.2	7.9
10	8.9
32	8.3
100	11.2

-Cumulative numbers of juveniles produced per adult

Nominal Conc. [mg/l]	Mean Cumulative Numbers of Juveniles Produced per Adult for 21 days 0-6	7	8	9	10	11	12	13	14
Control	0-0	1.0	11.8	12.0	14.0	36.5	36.6	43.0	66.4
3.2	0-0	4.8	10.6	11.0	24.0	37.6	37.6	50.1	67.2
10	0-0	0.1	8.3	9.0	9.9	32.6	32.8	39.8	63.5
32	0-0	3.7	8.8	8.8	13.3	30.6	30.7	41.4	58.3
100	0-0	0	0	0.3	0.8	2.7	4.1	4.1	5.6

Nominal Conc. [mg/l]	Mean Cumulative Numbers of Juveniles Produced per Adult for 21 days 15	16	17	18	19	20	21
Control	66.4	74.2	105.9	106.1	111.5	140.0	140.0
3.2	67.2	82.4	106.5	106.5	116.9	146.0	146.0
10	63.5	70.3	103.8	103.9	109.5	142.0	142.0

32	58.3	72.4	94.1	94.1	115.4	136.1	136.1
100	7.5	8.9	9.5	11.8	11.8	11.8	11.8

-Cumulative numbers of juveniles produced per adult alive for 21 days

Nominal Concentration (mg/L) (Measured Concentration, mg/L)					
Vessel No.	Control (2.83) (9.00)	3.2 (30.2)	10 (95.5)	32	100
1	162	150	D	140	13
2	151	136	147	147	12
3	152	143	125	114	5
4	142	150	D	168	12
5	150	154	139	125	11
6	136	124	151	125	12
7	128	152	152	142	10
8	118	140	129	129	17
9	121	162	140	149	11
10	140	149	153	122	15
Mean	140.0	146.0	142.0	136.1	11.8
S.D.	14.4	10.7	10.7	16.1	3.2
Inhibition ratio (%)		-4.3	-1.4	2.8	91.6
Significant difference		N.S.	N.S.	N.S.	*

D : Were not calculated because the parental Daphnia magna was dead during a 21-days testing period.
N.S. : Indicate a no-significant difference by Dunnett multiple comparison procedure.
* : Indicate a significant difference by Dunnett multiple comparison procedure .

- Calculation of toxicity values: The calculation of toxicity values was the Time weighted mean of measured concentrations.

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint

28-FEB-2005 (54)

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

4.6.3 Toxicity to Soil Dwelling Organisms

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: no data
Sex: male/female
No. of Animals: 5
Vehicle: water
Doses: 200, 1600, 3200, 4000, 5000, 6400 mg/kg bw
Value: ca. 3150 - 3850 mg/kg bw

Method: other: BASF-Test
Year: 1963
GLP: no
Test substance: other TS: 70% choline chloride in water, no further data

Result:

Dose in mg/kg bw	Mortality in males	in females
200	0/5	0/5
1600	0/5	0/5
3200	0/5	2/5
4000	0/5	2/5
5000	1/5	3/5
6400	5/5	5/5

Male rats:
LD50 ca. 5500 mg/kg bw related to 70% choline chloride.
Related to pure choline chloride: LD50 ca. 3850 mg/kg bw.
Female rats:
LD50 ca. 4500 mg/kg bw related to 70% choline chloride.
Related to pure choline chloride: LD50 ca. 3150 mg/kg bw.

Most rats died 8-60 min after application; one male rat died ca. 1.5 h after application (at a dose of 5000 mg/kg bw) and one female rat was found dead on the next morning (3200 mg/kg bw). The surviving rats showed a slight apathy on the day of application but the next day no clinical effects were observed.

Clinical symptoms after application: restlessness (starts 5-20 min after treatment), increased frequency of respiration, staggered gait, convulsions, side position, dyspnea.

Necropsy of rats found dead: reddened small intestine in 1 female after 3200 mg/kg bw; pale spleen (3 rats of the high dose group, 1 rat at 5000 mg/kg bw) or pale liver (1 rat at 5000 mg/kg bw).
Necropsy of rats sacrificed after 7 days: no effects.

Test condition: Application of 2% (200 mg/kg bw; 10 ml/kg bw), 20% (1600 mg/kg bw; 8 ml/kg bw), or 30% (3200-6400 mg/kg bw; 10.6-21.3 ml/kg bw) solution in aqua dest. (further dilution of the TS, 70% choline chloride in water); 5 male and 5

female rats ("Heigl" rats) per dose; initial body weight in females 142-196 g and in males 150-245 g; post exposure observation period 7 d; necropsy performed.

Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Restrictions: short post exposure observation period, no statistics

Flag: Critical study for SIDS endpoint

08-OCT-2004 (55)

Type: LD50
Species: rat
Strain: no data
Sex: male/female
No. of Animals: 10
Vehicle: other: aqueous suspension with Traganth
Doses: 200, 1600, 3200, 6400, 8000, 10000 mg/kg bw
Value: ca. 5000 mg/kg bw

Method: other: BASF-Test
Year: 1969
GLP: no
Test substance: other TS: "Choline chloride 50% powder": 50% choline chloride, 21% water, 29% colloidal silicic acid

Result:

Dose	Mortality	
in mg/kg bw	in males	in females
200	0/10	0/10
1600	0/10	0/10
3200	0/10	0/10
6400	0/10	2/10
8000	0/10	0/10
10000	3/10	5/10

Male rats:
LD50 > 10000 mg/kg bw related to 50% choline chloride powder.
Related to pure choline chloride: LD50 > 5000 mg/kg bw.
Female rats:
LD50 ca. 10000 mg/kg bw related to 50% choline chloride.
Related to pure choline chloride: LD50 ca. 5000 mg/kg bw.

Mortalities occurred the day after application with exception of 3 females which died ca. 5 h after treatment.

Clinical symptoms
after application of 6400-10000 mg/kg bw: hypoactivity immediately after exposure; increased frequency of respiration; ruffled, wet, and dirty coat; no effects detected 4-6 days after treatment.
200-3200 mg/kg bw: hypoactivity and ruffled coat; no effects observed after 3-5 days.

Necropsy of rats found dead: smudged anus and muzzle, diarrhoea.
Necropsy of rats sacrificed after 7 days: inflammation of the lung (1 rat at 6400 mg/kg bw and 2 rats at 10000 mg/kg bw); no further effects.

Test condition: Application of 2% (200 mg/kg bw; 10 ml/kg bw), 16% (1600 mg/kg bw; 10 ml/kg bw), or 30% (3200-6400 mg/kg bw;

10.6-33.3 ml/kg bw) aqueous suspension with Traganth; 10 male and 10 female rats ("Gassner" rats) per dose; initial body weight in females 138-200 g and in males 140-228 g; post exposure observation period 7 d; necropsy performed.
Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Restrictions: short post exposure observation period, no statistics
Flag: Critical study for SIDS endpoint
08-OCT-2004 (56) (57)

Type: LD50
Species: mouse
Strain: other: AB
Sex: male
No. of Animals: 5
Vehicle: water
Doses: At least 5 doses tested
Value: = 6000 mg/kg bw

Method: other
Year: 1974
GLP: no
Test substance: other TS: choline chloride, no further data

Result: Confidence limits: 5175-6840 mg/kg bw (p= 0.05)
Test condition: 5-10 male mice (18-24 g bw) per dose tested; post exposure observation period 7 days; LD50-value determined according to Litchfield and Wilcoxon (JPET 96, 99, 1949) using the data on 5-10 doses.
Reliability: (4) not assignable
Documentation insufficient for assessment.
Restrictions: short post exposure observation period, no data about symptoms & necropsy. No data on doses tested.
11-MAY-2004 (58)

Type: LD50
Species: rat
Strain: no data
Sex: no data
Value: = 3400 mg/kg bw

Method: other
GLP: no data
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
No further data available
27-SEP-2004 (11) (23) (59)

Type: LD50
Species: rat
Strain: no data
Sex: no data
Value: = 6640 mg/kg bw

Method: other
GLP: no data
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
No further data available
13-JUN-2003 (60)

Type: LD50
Species: mouse
Strain: no data
Sex: no data
Value: = 3900 mg/kg bw

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
No further data available
13-JUN-2003 (59)

5.1.2 Acute Inhalation Toxicity

Type: other: Inhalation Hazard Test
Species: rat
Strain: no data
Sex: male/female
No. of Animals: 12
Doses: 1.6 mg/l
Exposure time: 8 hour(s)

Method: other: BASF-Test
Year: 1969
GLP: no
Test substance: other TS: "Choline chloride 50% powder": 50% choline chloride, 21% water, 29% colloidal silicic acid

Method: Inhalation hazard test (rat):
This test (also called IRT) was performed in principle as described in the Annex to OECD Guideline 403 of May 12th, 1981.
It demonstrates the toxicity of an atmosphere saturated with vapours of the volatile components of a test substance at the temperature chosen for vapour generation (usually 20°C).
Young adult laboratory rats were purchased from a breeder. In general, the source and strain of the animals were not documented
Several groups of usually 3 rats per sex were exposed sequentially to the vapors, generated by bubbling 200 l/h air through a substance column of about 5 cm above a fritted glass disc in a glass cylinder for different time periods (e.g. 3 min, 10 min, 1, 3 or 7 or 8 hours). The exposure time not causing lethality was usually tested twice.
No analytical determination of the atmosphere concentrations was performed. The nominal concentration usually can be calculated as quotient of the amount of test substance weight loss during the exposure, which is given in the raw data, and the amount of air used during the exposure.
Group-wise documentation of clinical signs was performed over

the 7- to 14- day study period. Body weight of groups was determined before the start of the study and at the end of the observation period in surviving animals.
The clinical signs and findings were reported in summarized form. More details can usually be inferred as mentioned for the acute oral studies.
The study allows for an estimate of the length of time required to cause severe toxic effects resulting from exposure to an atmosphere saturated with volatile components of the test substance. The exposure time causing 50% lethality (LT50) can be estimated from such a study as described for the LD50. Furthermore, using the nominal concentration, vapour pressure and LT50, in many cases a 4-hour LC50 can be estimated using Haber's law.

Remark: Relevant deviations from a standard OECD TG403 study are:

Shorter Post-exposure observation time.
Fewer animals per concentration level (three of each sex instead of 5), but test was performed twice.
Only one test concentration (highest attainable under test conditions).
Longer exposure time (8 instead of 4 hrs; but Guideline allows for deviations from the usual 4 hrs).
No particle size determination due to exposure of volatile parts of the test substance. Therefore no dust generation.

Result: No mortality in 12 exposed rats; no symptoms recorded during and after exposure; necropsy: no effects.

Test condition: For saturation of the atmosphere air conducted through a layer of the tested product (height 5 cm; 200 l air per h); test at 20°C; concentration of the TS: 1.6 mg/l (concentration estimated by determination of TS weight before and after exposure period; no dust); post exposure observation period 8 days; 2 independent trials (n=6 each trial; male and female rats, no further data); necropsy performed with no findings.

Reliability: (3) invalid
Unsuitable test system for salts with low vapour pressure or aqueous solutions thereof

08-OCT-2004 (61)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rat
Strain: no data
Sex: no data
No. of Animals: 5
Vehicle: other: undiluted TS applied
Doses: 2 ml/rat
Value: > 9.7 ml/kg bw

Method: other: see test condition
GLP: no
Test substance: other TS: aqueous solution; choline chloride 70%, 30% water

Result: No mortality; no clinical effects observed during and after exposure; also no local effects (no irritation); necropsy: no macroscopic effects detected in any organ.
LD50 > 10700 mg/kg bw; related to the pure TS LD50 > 7500

mg/kg bw.
Test condition: 2 ml of the undiluted TS given into a bathtub; rats (n=5) with shaved abdomen placed in the bathtub and exposed for 4 h; after exposure skin washed with Lutrol;
exposed area of the skin: 15-24 cm^2;
body weight of the rats: 116-206 g;
post exposure observation period 4 weeks; necropsy.
Reliability: (3) invalid
Unsuitable test system

13-JUN-2003 (62)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: mouse
Strain: no data
Sex: male/female
No. of Animals: 5
Vehicle: other: see freetext
Doses: 200, 320, 400, 500, 640, 800, 1600 mg/kg bw
Route of admin.: i.p.
Value: ca. 500 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS: "Choline chloride 50% powder": 50% choline chloride, 21% water, 29% colloidal silicic acid

Remark: No further data available
Result: LD50 for males and females combined ca. 500 mg/kg bw related to 50% choline chloride powder.
LD50 of the pure TS ca. 225 mg/kg bw.
Mice died within 2 min (high dose) or within 1 h after injection (640-800 mg/kg bw; no mice survived); at 500 mg/kg bw 3 mice were found dead the next day (1. trial, 1/5 m & 2/5 f) or all mice died within the 1st 10 min (2. trial, 5 m & 5 f).
Symptoms (at >= 320 mg/kg bw): immediately after injection abdominal position, increased frequency of respiration, convulsions, dyspnoea, exophthalmus, cyanosis. Slight effects also after 200 mg/kg.
Necropsy: occasional adhesions in the area of the liver
Test condition: Application of 2, 4, 8, or 16% aqueous suspension with Traganth. At least 5 male and 5 female mice per dose; post exposure observation period 7 d; necropsy performed.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
08-OCT-2004 (63)

Type: LD50
Species: rat
Strain: other: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: i.p.

Value: = 450 mg/kg bw

Method: other: no data
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
12-MAY-2004 (59)

Type: LD50
Species: rat
Strain: no data
Sex: male
Vehicle: no data
Doses: 378-532 mg/kg bw
Route of admin.: i.p.
Value: = 450 mg/kg bw

Method: other
Year: 1986
GLP: no
Test substance: other TS: choline chloride, no further data

Remark: No further data available.
Result: Range: 378-532 mg/kg bw
Reliability: (4) not assignable
Documentation insufficient for assessment
12-MAY-2004 (64)

Type: LD50
Species: mouse
Strain: no data
Sex: male/female
No. of Animals: 5
Vehicle: water
Doses: 25, 200, 250, 320, 400, 800, 1600 mg/kg bw
Route of admin.: i.p.
Value: ca. 350 mg/kg bw

Method: other: BASF-Test
Year: 1963
GLP: no
Test substance: other TS: 70% choline chloride in water, no further data

Result: LD50 for male and female mice combined ca. 350 mg/kg bw related to 70% choline chloride.
LD50 of the pure TS ca. 240 mg/kg bw.
Symptoms (at >= 250 mg/kg bw): restlessness, increased frequency of respiration, staggered gait, convulsions, side position, dyspnoea. Mice died within 2-5 min.
Necropsy: no effects detected in sacrificed mice; mice found dead showed increased fluid in the peritoneum.
Test condition: Application of 0.2, 2, 8, or 20% aqueous solution. 5 male and 5 female mice per dose; post exposure observation period 7 d; necropsy performed.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint

12-MAY-2004 (65)

Type: LD50
Species: mouse
Strain: other: AB
Sex: male
No. of Animals: 5
Vehicle: water
Doses: at least 5 doses tested
Route of admin.: i.p.
Value: = 300 mg/kg bw

Method: other
Year: 1974
GLP: no
Test substance: other TS: choline chloride, no further data

Result: Confidence limits: 250-360 mg/kg bw (p= 0.05)
Test condition: 5-10 male mice (18-24 g bw) per dose tested; post exposure observation period 7 days; LD50-value determined according to Litchfield and Wilcoxon (JPET 96, 99, 1949) using the data on 5-10 doses.
Reliability: (4) not assignable
Documentation insufficient for assessment
12-MAY-2004 (58)

Type: LD50
Species: mouse
Strain: no data
Sex: no data
Vehicle: no data
Route of admin.: i.p.
Value: = 320 mg/kg bw

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
12-MAY-2004 (59)

Type: LDLo
Species: rabbit
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: i.p.
Value: = 500 mg/kg bw

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
12-MAY-2004 (59)

Type: LDLo

Species: mouse
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: s.c.
Value: = 735 mg/kg bw

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
12-MAY-2004 (59)

Type: LDLo
Species: rabbit
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: s.c.
Value: = 1000 mg/kg bw

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
12-MAY-2004 (59)

Type: LD50
Species: mouse
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: i.v.
Value: = 53 mg/kg bw

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
12-MAY-2004 (59)

Type: LD50
Species: mouse
Strain: other: AB
Sex: male
No. of Animals: 5
Vehicle: water
Doses: at least 5 doses tested
Route of admin.: i.v.
Value: = 49 mg/kg bw

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Result: Confidence limits: 44-55 mg/kg bw (p= 0.05)
Test condition: 5-10 male mice (18-24 g bw) per dose tested; post exposure observation period 7 days; LD50-value determined according to Litchfield and Wilcoxon (JPET 96, 99, 1949) using the data on 5-10 doses.

Reliability: (4) not assignable
Documentation insufficient for assessment
12-MAY-2004 (58)

Type: LDLo
Species: rabbit
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: i.v.
Value: = 1.1 mg/kg bw

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
12-MAY-2004 (59)

Type: LDLo
Species: cat
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: i.v.
Value: = 25 mg/kg bw

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
12-MAY-2004 (59)

Type: LDLo
Species: dog
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: i.v.
Value: = 5 mg/kg bw
Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature

12-MAY-2004 (59)

Type: LDLo
Species: rabbit
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: other: rectal
Value: = 1000 mg/kg bw

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
12-MAY-2004 (59)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration: undiluted
Exposure: Occlusive
Exposure Time: 20 hour(s)
No. of Animals: 2
PDII: .13
Result: slightly irritating
EC classificat.: not irritating

Method: other: BASF-Test
Year: 1963
GLP: no
Test substance: other TS: 70% choline chloride in water, no further data

Remark: Compared to OECD TG 404, Acute Dermal Irritation/Corrosion the test protocol used was significantly harsher: Exposure time was 20 hrs instead of 4 hrs with occlusive instead of semi-occlusive dressing. This protocol has a tendency to overestimate the skin irritating potential of a substance. There is no major difference in the concentrations applied as compared to the current guideline protocol.
No further data available.
Result: Questionable reddening after 24 h only in one rabbit (on the back; no irritation on the ear); no further effects detected.
Test condition: 2 female rabbits used (white Viennese; initial weight 2.43 or 3.05 kg); a 2.5 x 2.5 qcm gauze patch was soaked with 2 ml of the undiluted TS. The gauze was applied to the shaved dorsal skin of the rabbit and covered with occlusive dressing.

Exposure time was for 20 h with readings

24 h, 2 d, 3 d or 8 d after application.
Attached doc.: BASF testing before existing OECD TGs.pdf
Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions
Flag: Critical study for SIDS endpoint
28-FEB-2005 (66)

Species: rabbit
Concentration: 50 %
Exposure: Occlusive
Exposure Time: 20 hour(s)
No. of Animals: 2
Vehicle: water
PDII: .3
Result: slightly irritating
EC classificat.: not irritating

Method: other: BASF-Test
GLP: no
Test substance: other TS: "Choline chloride 50% powder": 50% choline chloride, 21% water, 29% colloidal silicic acid

Remark: Colloidal silicic acid was used as a carrier for the test substance. According to the technical process how colloidal silicic acid is made as a water soluble sodium silicate with a progressive release of silicium dioxide under more acidic conditions, it cannot be ruled out that under the occlusive test conditions used in the current study protocol some alkali with a proportionate irritating effect was generated. As a potential irritating effect by the pure colloidal silicic acid was not determined under the same study conditions, the result of this study is regarded as invalid.
Compared to OECD TG 404, Acute Dermal Irritation/Corrosion the test protocol used was significantly harsher: Exposure time was 20 hrs instead of 4 hrs with occlusive instead of semi-occlusive dressing. This protocol has a tendency to overestimate the skin irritating potential of a substance. There is no major difference in the concentrations applied as compared to the current guideline protocol.

Result: Exp. design 1)
- no irritation detected after 1 or 5 min exposure
- after 15 min exposure questionable reddening was observed 2 and 5 days after application in one rabbit and the other animal showed a questionable reddening only 2 days after treatment.

Exp. design 2)
- slight reddening on the 2 application sites of both rabbits 1 and 2 days after application, skin appeared marked
- 5 days after treatment one rabbit showed questionable fine scales, no effects detected in the other animal
- no effects in both animals after 8 d.

Test condition: 50% aqueous suspension of the TS applied to the skin of 2 female rabbits (white viennese; initial weight 2.02 kg and 2.13 kg, final weight 7 days after application 2.00 and 2.21 kg, respectively).

Exp. design 1)
- 50% suspension (volume not given) applied to 3 different

sites (anterior, median, posterior part) of the right (rabbit No. 1) or the left back (rabbit No.2)
- skin washed after 1 min, 5 min or 15 min exposure time, respectively, with undiluted Lutrol and 50% solution of Lutrol (no further data)
- readings performed on the day of application, and 1, 2, 5, 7 days after treatment

Exp. design 2)
- same animals received the 50% suspension (volume not given) to the median part of the left back (rabbit No. 1) or the right back (No.2) and additionally to the skin of the left ear; exposure time 20 h (no further data)
- effects scored 1, 2, 5, 7 days after application

Attached doc.: BASF testing before existing OECD TGs.pdf
Reliability: (3) invalid
Significant methodological deficiencies
28-FEB-2005 (67)

Species: rat
Concentration: undiluted
Exposure: Open
Exposure Time: 4 hour(s)
No. of Animals: 5
Vehicle: water
Result: not irritating
EC classificat.: not irritating

Method: other: see freetext
Year: 1963
GLP: no
Test substance: other TS: aqueous solution; choline chloride 70%, 30% water%

Remark: No data about number of observations and observation time.
Result: No local effects observed.
Test condition: 2 ml of the undiluted TS given into a bathtub; rats (n=5) with shaved abdomen placed in the bathtub and exposed for 4 h; after exposure skin washed with Lutrol;
exposed area of the skin: 15-24 cm^2;
body weight of the rats: 116-206 g;
post exposure observation period 4 weeks; necropsy
Reliability: (4) not assignable
Documentation insufficient for assessment
28-FEB-2005 (62)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .5 ml
Exposure Time: unspecified
Comment: other: presumably not rinsed
No. of Animals: 2
Vehicle: none
Result: slightly irritating
EC classificat.: not irritating

Method: other: BASF-Test
Year: 1963

GLP: no
Test substance: other TS: 70% choline chloride in water, no further data

Remark: No further data available.
Result: Male rabbit, right eye:
slight reddening and increased secretion (tears) detected after 10 min, slight reddening after 1 and 3 h, but no irritation observed after 1 d or later; no effects detected on the cornea.
According to the raw data the reddening had a score of 1.
Female rabbit, right eye: increased secretion after 10 min; no effects recorded after 1 h, but questionable effects (presumably reddening) after 3 h; no effects detected after 1 d or later; cornea: no effects.
In both rabbits no effects seen in the left eye (control).
According to the raw data the reddening had a score of 1.
Test condition: 1 male (initial body weight 2.57 kg) and 1 female rabbit (initial body weight 2.51 kg) used; 1 droplet TS into the right eye and 1 droplet physiological saline into the left eye (control); readings 10 min, 1 h, 3 h, 1 d, 2 d (one animal) and 8 days after application.
Reliability: (2) valid with restrictions
Meets generally accepted standards, acceptable for assessment.
Flag: Critical study for SIDS endpoint
25-FEB-2005 (68)

Species: rabbit
Concentration: other: application of the powder
Dose: .5 ml
Comment: other: not rinsed
No. of Animals: 2
Vehicle: none
Result: slightly irritating
EC classificat.: not irritating

Method: other: BASF-Test
Year: 1969
GLP: no

Test substance: other TS: choline chloride 50% powder; 21% water, 29% silicic acid (colloidal) and 50% choline chloride

Remark: Possibly effects due to mechanical irritation (powder) when compared with choline chloride solution in water (BASF 1963). Colloidal silicic acid was used as a carrier for the test substance. According to the technical process how colloidal silicic acid is made as a water soluble sodium silicate with a progressive release of silicium dioxide under more acidic conditions, it cannot be ruled out that under the occlusive test conditions used in the current study protocol some alkali with a proportionate irritating effect was generated. As a potential irritating effect by the pure colloidal silicic acid was not determined under the same study conditions, the result of this study is regarded as invalid.
Result: RECORDED EFFECTS

	rabbit No.1		rabbit No.2	
after	TS	control	TS	control
10 min	R++, E+	R+	R+, E+	R+
1 h	R++, E+	R+	R+, E+	R+

3 h	R++, E+	R+	R+, E+	R+, E+
1 d	R+	0	R+	R+
2 d	R+	nd	0	nd
3 d	R+	nd	0	nd
4 d	0	nd	0	nd
7 d	0	0	0	0

R: reddening; E: oedema; +: slight effect; ++: strong effect; nd: no data; 0: no irritation

Remnants of the powder (or the talcum) detected up to 3 h after application. No effects detected on the cornea (after fluorescein application) 7 days (rabbit No.1) or 4 days (rabbit No.2) after treatment.

Test condition: Ca. 50 mm^3 choline chloride powder applied to the right eye of each rabbit (2 female animals, initial weight 2.62 or 2.57 kg; final weight 2.63 or 2.65 kg, respectively). The left eye served as control and was treated with 50 mm^3 talcum. Effects were scored 10 min, 1 h, 3 h, and 1, 2, 3, 4, and 7 days after application.

Reliability: (3) invalid
Significant methodological deficiencies

28-FEB-2005 (69)

5.3 Sensitization

Type: other

Remark: See section 5.10

11-MAR-2003

5.4 Repeated Dose Toxicity

Type: Chronic
Species: rat **Sex:** male
Strain: Fischer 344
Route of administration: oral feed
Exposure period: 72 weeks
Frequency of treatment: daily ad libitum
Post exposure period: 31 weeks
Doses: 1% in the diet (ca. 500 mg/kg bw/day)
Control Group: yes, concurrent vehicle

Method: other: see freetext
GLP: no
Test substance: other TS: choline chloride, no further data

Remark: No further data available.
Result: No significant differences between control group and treated animals concerning body weight (week 10: 258 g versus 253 g in control; week 50: 406 g versus 408 g), survival at week 52 (28 versus 28), at week 78 (28 versus 28), at week 102 (24 versus 23), relative liver weight (3.4% versus 3.6%), neoplastic liver nodules (incidence: in 2 treated rats versus in 2 control rats), and hepatocellular carcinomas (incidence: 0 versus 1). No increase in the incidence of lung tumors,

leukaemia or other tumours (no further specification).
Especially, dietary feeding of methionine and and choline choride either alone or in combination with phenobarbital or DDT did not have any significant effect on the incidence of hepatocellular carcinomas.
Liver tumor formation was negligible in uninitiated rats.

Test condition: The effect of chronic feeding of choline chloride on liver tumour promotion was studied in rats receiving an initiating dose of diethyl nitrosamine (DEN).
30 male weanling rats (initial weight 50-60 g) per treatment group were injected i.p. with 200 mg/kg bw.
Control rats were injected with saline.
Five days after injection, the rats were placed on different diets containing 0.05% of phenobarbital or 0.05% of 1,1 bis(p-chlorophenyl)-2,2,2-trichlorethane (DDT) with or without added 1.5% DL-methionine or 1.0% choline chloride.
One control group was also initiated with DEN and then treated with choline chloride (1.0%) in the absence of any other tumour promoter.
Each diet was administered for 72 weeks, when the animals were placed on the unsupplemented chow diet for an additional 30 weeks.

Body weight was determined weekly for the first 16 weeks and then biweekly;
necropsy at week 103; histopathology limited to the liver and "organs bearing gross abnormalities".

Reliability: (2) valid with restrictions

Meets generally accepted standards, acceptable for assessment.
Restrictions: one dose tested, limited histopathology.

Flag: Critical study for SIDS endpoint

08-OCT-2004 (70)

Type: Sub-chronic
Species: rat **Sex:** male
Strain: no data
Route of administration: i.p.
Exposure period: exp. design 1: 5 weeks; exp. design 2: 8 weeks
Frequency of treatment: 1) once daily, 5 d/week, total 24 injections; 2) once daily, 5 d/week, total 40 injections
Post exposure period: 1) 1, 3, or 8 months; 2) 3 or 6 months
Doses: 1) 0, 45, 148, 225 mg/kg bw (LD50= 450 mg/kg bw); 2) 148 mg/kg bw
Control Group: other: concurrent vehicle control in 1) but presumably no control in 2) (see freetext)

Method: other: see freetext
GLP: no
Test substance: other TS: choline chloride, no further data

Result: EXP. DESIGN 1
- initial excitement in all animals observed which lasted for 5 min; then rats became dull and sluggish (no data about dose response);
- a few animals died during the experiment (no further data);
- no significant differences in body weight gain except a significant increase in the mid dose group after 3 months

post exposure observation period;
- rel. lung weight was significantly decreased after 1 month in all treatment groups, but significantly increased in the high dose group after 3 months;
- the rel. liver and thymus weight was significantly decreased after 1 month in the high dose group, after 8 months the thymus weight was also significantly decreased in the mid and high dose group;
- the rel. peripheral lymph node weight was significantly increased in the mid dose group after 3 months;
- in all groups cell counts were significantly and dose dependently decreased in thymus and peripheral lymph nodes after 8 months, effects were also seen in the spleen, but there was no dose dependency; after 1 month the cell counts in the peripheral lymph nodes were significantly increased (dose dependent)

EXP. DESIGN 2 (histopathology)
- histopathology of the lung revealed hyper reactive bronchiolar epithelium and adenomatoid changes after 3 and 6 months with increasing collagen and reticulin fibres after 6 months (no data about the dose-effect-relationship);
  the lymph nodes showed loss of normal architecture (no data about the dose);
  swollen Kupffer cells were detected in the liver after 3 months and after 6 months necrosis together with lymphocytic infiltration (no data about dose dependency).

Test condition: EXPERIMENTAL DESIGN 1
- initially 24 rats per dose;
- after treatment period 5 rats per dose per post exposure observation period used for determination of the following parameters: body weight gain, b) organ weight (restricted to lung, liver, kidney, spleen, thymus, adrenal gland and lymph nodes of different sites) after autopsy, c) number of nucleated cells in the cell suspension of the organs (thymus, spleen, lymph nodes).

EXPERIMENTAL DESIGN 2
- initially 10 rats received TS treatment for 8 weeks "and sacrificed at 3 and 6 months" (no further data); only histopathology performed in this additional group; no data about control in the section "material and methods".
- In the result section data on control rats mentioned, but control not specified.

Reliability: (4) not assignable
Reliability unassignable

08-OCT-2004 (64)

Type: Sub-chronic
Species: guinea pig **Sex:** male
Strain: no data
Route of administration: i.p.
Exposure period: 8 weeks
Frequency of treatment: once daily, 5 days per week
Post exposure period: 570 or 680 days
Doses: 50 mg/animal (ca. 150 mg/kg bw)
Control Group: other: type of control not specified

Method: other: see freetext
GLP: no

Test substance: other TS: choline chloride, no further data

Remark: Irrelevant route of exposure, shortcomings of documentation and limited histopathology

Result: After 570 days cellular mass outside the lung lobe containing hyperchromatic cells and attached to the lung by connecting cellular band were detected (no quantification given);
at 680 days dysplasia of the mucosa with occasional keratinisation of the bronchiolar epithelium was observed and large amounts of pigments were found in the medullary region of the lymph-nodes (also no quantification of these effects); no effects were seen in controls.

Test condition: 10 animals i.p. injected daily with the TS in 2 ml sterile aqua dest. for 8 weeks; presumably 5 animals per post exposure observation period; animals sacrificed and lungs including tracheobronchial lymph-nodes prepared for histopathology; no further parameter studied; 5 control animals (no data about type of control and number of animals in each post exposure observation period group).

Reliability: (4) not assignable
Reliability unassignable

08-OCT-2004 (71)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537
Concentration: Lab 1: 0, 333, 1000, 3333, 10000, 20830 µg/plate; Lab 2 & 3: 0, 100, 333, 1000, 3333, 10000 µg/plate
Cytotoxic Concentration: no cytotoxicity in preliminary tests at dose levels up to 10 mg/plate; no cytotoxicity concerning decrease in revertants in the main study (exception in 1 out of 3 labs, see freetext); max. dose sufficient (see OECD 471)
Metabolic activation: with and without
Result: negative

Method: other: comparable to OECD Guide-line 471
GLP: no
Test substance: other TS: choline chloride, no further data

Result: CYTOTOXICITY:
- slight decrease in the number of revertants at the high dose of 10 mg/plate in one laboratory (TA1535, TA1537); no cytotoxicity in the other two labs.

GENOTOXIC EFFECTS:
- With and without metabolic activation no increase in revertants at any dose level in all tested strains.

CONTROLS:
- spontaneous revertants in negative controls within the normal range; valid positive controls.

Evaluation:
Under the condition of this study the TS did not cause an increase in the number of revertants of any tester strain either with or without metabolic activation

Test condition: SYSTEM OF TESTING

- Type: preincubation procedure
- 2 different metabolic activation (MA) systems; S9-mix, liver microsomes prepared from 1) male Sprague-Dawley rats and from 2) male Syrian hamsters; both pretreated with i.p. 500 mg/kg bw Aroclor1254
- 2 independent trials in each of 3 different laboratories, 3 plates per dose/exp. design
- Solvent: dest. water
- Negative controls: solvent used
- Positive controls without MA:
  TA98 3.3-12 µg/plate 4-nitro-o-phenylenediamine
  TA100 and TA1535 1.0-3.3 µg/plate sodium azide
  TA1537 33-80 µg/plate 9-aminoacridine
- Positive controls with MA:
  all tested strains 0.75-2.5 µg/plate 2-aminoanthracene
- Cytotoxicity: tested in preliminary studies on TA100; bacteria incubated at concentrations up to 10 mg/plate with and without MA; no cytotoxicity observed (decrease in bacterial lawn or number of revertants)

CRITERIA FOR EVALUATING RESULTS:
considered positive if the TS produced a dose related increase in revertants (not restricted to a 2-fold increase in revertants per plate over vehicle control).

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

Flag: Critical study for SIDS endpoint

13-JUN-2003 (72) (73)

Type: Ames test
System of testing: Salmonella typhimurium TA98, TA100, TA 1535, TA1537, TA1538
Concentration: 0, 1.25, 2.5, 5% (0, 12.5, 25, 50 mg/ml)
Cytotoxic Concentration: high dose resulted in 50% survival of bacteria
Metabolic activation: with and without
Result: negative

Method: other: comparable to OECD Guide-line 471
GLP: no
Test substance: other TS: choline chloride, white crystals, no further data

Result: GENOTOXIC EFFECTS:
- With and without metabolic activation no increase in revertants at any dose level in all tested strains.

CONTROLS:
- spontaneous revertants in negative controls within the normal range; valid positive controls.

Evaluation:
Under the condition of this study the TS did not cause an increase in the number of revertants of any tester strain either with or without metabolic activation.

Test condition: SYSTEM OF TESTING
- Type: 1) plate incorporation method and 2) suspension method (1 h exposure)
- 6 different metabolic activation (MA) systems; S9-mix, liver or lung microsomes prepared from 1) male Sprague-Dawley rats, 2) male ICRFLO mice, 3) male rhesus monkey (all species without pretreatment)
- 1 trial per exp. design

- Solvent: phosphate buffer
- Negative control: solvent used
- Positive controls without MA:
  TA98 and TA1538 100 µg/plate 2-nitrofluorene
  TA100 and TA1535 2 µg/plate methylnitrosoguanidine
  TA1537 20 µg/plate quinacrine mustard
- Positive controls with MA:
  TA98 and TA1538 100 µg/plate 2-acetylaminofluorene
  TA100 and TA 1535 100 µg/plate 2-aminoanthracene
  TA1537 100 µg/plate 8-aminoquinoline
- Cytotoxicity: tested in preliminary studies; bacteria incubated at 37°C for 1 h with 0.0005, 0.005, 0.05, 0.5, 5% TS in buffer

CRITERIA FOR EVALUATING RESULTS:
considered positive if the TS produced at least a 2-fold increase in revertants per plate over vehicle control and a dose response to increasing concentrations

Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Restrictions: no repeat trials

Flag: Critical study for SIDS endpoint

13-JUN-2003 (74)

Type: Ames test
System of testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537; E. coli WP2 uvrA
Concentration: 0.0763, 0.305, 1.22, 4.88, 19.5, 78.1, 313, 1250, 5000 µg/plate
Cytotoxic Concentration: no cytotoxicity concerning decrease in revertants; max. dose of 5 mg/plate sufficient (see OECD 471)
Metabolic activation: with and without
Result: negative

Method: other: Japanese Industry and Health Law Article 57-2 §1
Year: 1988
GLP: no
Test substance: other TS: choline chloride, purity >= 99%

Result: GENOTOXIC EFFECTS
- With and without metabolic activation (MA) revertants per plate similar to control values at all dose levels in all tested strains in 2 trials; high dose in TA98 without MA resulted in a decrease of revertants (7/plate versus 13/plate in control.

CONTROLS
- spontaneous revertants in negative controls within the normal range;
- valid positive controls.

EVALUATION
- Under the condition of this study the TS did not cause an increase in the number of revertants of any tester strain either with or without metabolic activation.

Test condition: SYSTEM OF TESTING

- Metabolic activation (MA) system: S9-mix, liver microsomes
- 2 independent trials per concentration
- Solvent: aqua dest. (TS soluble)
- Negative controls: solvent

- Positive control without MA:
  TA98, TA100, and WP2 2-aminofluorene
  TA1535 sodium azide
  TA1537 9-aminoacridine
- Positive control with MA
  in all strains 2-aminoanthracene used
- Cytotoxicity: evaluated via reduction in revertant colonies

Reliability: (2) valid with restrictions
Meets national standard methods with acceptable restrictions
Restrictions: no data about bacterial background lawn
Flag: Critical study for SIDS endpoint
13-JUN-2003 (75)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: 2 independent studies: 0.005-500 µg/ml in Lab1 and 0.05-5000 µg/ml in Lab2
Cytotoxic Concentration: cytotoxic effects at the highest dose (see also freetext)
Metabolic activation: with and without
Result: negative

Method: other: see freetext; comparable to OECD Guide-line 473
Year: 1984
GLP: no
Test substance: other TS: choline chloride supplied by NTP, no further data

Remark: In comparison to the OECD TG473 the following differences have been recorded for the present study:
- Instead of the recommended 200 metaphases per dose level only one hundred cells per dose level have been examined.
- While the guideline recommends an exposure time with and without metabolic activation for 3-6 hrs and a culture harvest time equivalent to 1.5 normal cell cycle length after the beginning of the experiment harvest time in the current study (including metabolic activation) has been shorter, i.e. 8-12 hrs (cells in the first metaphase).
- The guideline recommends a difference of 2 to the square root of ten between different doses starting down from the highest dose (showing significant cytotoxicity). The scaling factors in the current study have been 10 (to cover 5 orders of magnitude) in two experiments and a factor of 2 in one experiment covering the range from 1000 to 5000 µg/ml.
- The positive control without metabolic activation is not included in the test guideline recommendation for positive control substances. The test guideline allows for the choice of alternatives, however.

In spite of these restrictions the current study has correctly determined the positive controls with and without metabolic activation and is therefore considered to be valid.

Result: CLASTOGENIC EFFECTS in LAB1

	Aberrations in % without MA	
Dose in µg/ml	Simple	Complex
control	1	0
0.005	0	0
0.05	0	0
0.5	0	0
50	3	0
500	7	0

Only 500 µg/ml revealed a statistically significant positive result (trend positive). With MA no clastogenic activity.

CLASTOGENIC EFFECTS in LAB2
In 2 independent trials no clastogenic activity observed without MA at dose levels of 0.05, 0.5, 5, 50, 500, or 5000 µg/ml (1st trial) and 1000, 2000, 3000, 4000, 5000 µg/ml (2nd trial); also no clastogenic activity was detected in one trial with MA at dose levels of 0.05, 0.5, 5, 50, 500, 5000 µg/ml.

CONTROLS in LAB1 and 2
Valid positive (14-70% abnormal cells without MA and 20-45% with MA) and negative control.

CONCLUSION
No clastogenic activity (no clear conclusion given by the authors).

Test condition: PRETEST FOR DOSE SELECTION / CYTOTOXICITY
In preliminary tests cells exposed for 24 h to a series of doses covering 5 orders of magnitude; the highest dose used for the cytogenetic study was the concentration that reduced the cell number by about 50%. Dose selection was changed in the 2nd set of experiments: in the cytogenetic study the cytotoxicity was evaluated by examination of the cultures just before fixation (confluence of the monolayer, healthy mitotic cells) and cells of the highest dose level to yield analysable metaphases were fixed together with 5 successively lower concentration levels.

SYSTEM OF TESTING
- test procedure: cells sampled 8 or 12 h after starting the exposure with and without metabolic activation (cells in the 1st metaphase); colchicine added the last 2 hrs; in test with metabolic activation the S9 mix was present only the initial 2 hrs; microscopic examination on a blind basis; gaps and endoreduplications not included in the evaluation of aberrations; individual types of aberration recorded separately; evaluation of simple (breaks and terminal deletions) and complex (including exchanges and rearrangements, no further data) aberrations
- Metabolic activation (MA) system: S9-mix, liver microsomes prepared from male Sprague-Dawley rats treated with Aroclor1254
- number of cells examined: 100 cells/dose level,
- Solvent: supplemented McCoy's 5A medium (culture medium)
- Controls: negative (solvent control)
  positive control 0.25 µg/ml triethylene melamine (without MA) or 25 µg/ml cyclophosphamide (with MA)

CRITERIA FOR EVALUATING RESULTS:
- considered positive if the TS produced a significant, dose-related, reproducible increase in aberrations; trend test performed (Armitage, 1955).

Attached doc.: CA CHO study 794173.xls

CA CHO study 796173.xls
CA CHO study 870461.xls
Genetic Toxicity Study Options In Vitro Cytogenetics.htm
Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Flag: Critical study for SIDS endpoint
01-JUL-2004 (76) (77) (78)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: 0, 2000, 3000, 4000, 5000 µg/ml
Cytotoxic Concentration: no data
Metabolic activation: with and without
Result: negative

Method: other: see freetext; comparable to OECD Guide-line 473
GLP: no
Test substance: other TS: choline chloride, no further data

Result: CLASTOGENIC EFFECTS

	Aberrations in %			
Dose in µg/ml	Simple-MA	Complex-MA	Simple+MA	Complex+MA
control	1	0	2	1
2000	0	0		
3000	1	4	3	2
4000	2	2	2	3
5000	1	1	0	1
positive control	14	7	7	23

(no details about "simple" and "complex" aberrations given by the authors)

CONTROLS
Valid positive and negative control.

CONCLUSION:
No clastogenic activity.

Test condition: SYSTEM OF TESTING
- test procedure without MA: cells incubated for 8-10 h in the medium containing the TS; this medium was replaced by fresh medium containing colcemid, incubation for 2-3 h; cell harvested, fixed and stained with Giemsa.
- test procedure with MA: cells incubated in serum-free medium containing the TS and S9-mix; further incubation for 8-10 h in fresh medium without TS and S9-mix, colcemid present the last 2-3 h; further preparation see above.
- Metabolic activation (MA) system: S9-mix, liver microsomes

  prepared from male Sprague-Dawley rats treated with Aroclor1254
- number of cells examined: 100 cells/dose level,
- Solvent: supplemented McCoy's 5A medium (culture medium)
- Controls: negative (solvent control)
  positive control mitomycin C (without MA) or cyclophosphamide (with MA)

CRITERIA FOR EVALUATING RESULTS:
- no data

Attached doc.: CA CHO study 794173.xls
CA CHO study 796173.xls
CA CHO study 870461.xls
Genetic Toxicity Study Options In Vitro Cytogenetics.htm
Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Restrictions: no repeat trials, no data about cytotoxicity but highest dose similar to dosing in other valid studies.
Flag: Critical study for SIDS endpoint
01-JUL-2004 (78)

Type: Sister chromatid exchange assay
System of testing: Chinese hamster ovary cells
Concentration: 2 independent studies: 0.005-500 µg/ml in Lab1 and 0.05-5000 µg/ml in Lab2
Cytotoxic Concentration: cytotoxic effects at the highest dose (see also freetext)
Metabolic activation: with and without
Result: ambiguous

Method: other: see freetext, comparable to OECD Guide-line 479
GLP: no
Test substance: other TS: choline chloride supplied by NTP, no further data

Result: RESULTS in LAB1 and LAB2
Because of an insufficient number of control cells in LAB2 in the trial without MA these data were not analysable.

	Number of SCEs per cell		
Dose in µg/ml	LAB1 -MA	LAB1 +MA	LAB2 +MA
control	7.09	7.23	7.07
0.005	6.77	7.72	
0.05	6.59	7.66	8.75*
0.5	7.78	8.23	8.30
5			9.37*
50	8.32	8.18	8.03
500	8.73*	8.64	7.81
5000			7.90
trend	P<0.005	P<0.025	
evaluation	questionable positive	weak positive	positive without dose dependency

*: 20% more SCEs than control

CONTROLS in LAB1 and LAB2
Valid positive (23-63 SCEs/cell without MA and 25-45 SCEs/cell with MA) and negative control (exception see above).

CONCLUSION
authors' comment: the slight positive indications for the SCE test require confirmation.
Test condition: PRETEST FOR DOSE SELECTION / CYTOTOXICITY
In preliminary tests cells exposed for 24 h to a series of doses covering 5 orders of magnitude; the highest dose used for the cytogenetic study was the concentration that reduced the cell number by about 50%. Dose selection was changed in the 2nd set of experiments: in the cytogenetic study the cytotoxicity was evaluated by examination of the cultures

just before fixation (confluence of the monolayer, healthy mitotic cells) and cells of the highest dose level to yield analysable metaphases were fixed together with 5 successively lower concentration levels.

SYSTEM OF TESTING
- test procedure without MA: cells exposed to the TS for 2 h without addition of BrdU; then 10 µM BrdU added and exposure continued for 24 h; after washing cells incubated in medium containing 10 µM BrdU and 0.1 µg/ml colcemid for 2-3 h; cells were then collected by the mitotic shake-off method, treated for up to 3 min with hypotonic KCl, washed twice with fixative and air-dried on slides; staining according to modified fluorescence plus Giemsa technique (slides stained for 10 min with Hoechst33258 in phosphate buffer, mounted in the same buffer and exposed at 55-65°C to "blacklight" for 3-8 min, finally slides stained with Giemsa and air-dried); 50 M2 cells (completed 2 cell cycles) per dose scored for SCEs.

- test procedure with MA: addition of S9-mix to the medium plus TS, incubation for 2 h followed by washing; then cells incubated in medium containing 10 µM BrdU and 10% fetal calf serum for 26 h, with colcemid present the last 2-3 h; further preparation see above.

- Metabolic activation (MA) system: S9-mix, liver microsomes prepared from male Sprague-Dawley rats treated with Aroclor1254

- number of chromosomes examined: ca. 1050 per dose level,

- Solvent: supplemented McCoy's 5A medium (culture medium)

- Controls: negative (solvent control)
positive control 15 ng/ml triethylenemelamine (without MA) or 1.5 µg/ml cyclophosphamide (with MA)

- one trial in each laboratory performed

CRITERIA FOR EVALUATING RESULTS:
- considered positive if the TS produced a significant, dose-related, reproducible increase in SCE/cell of more than 20%; statistical analysis according to Armitage (Biometrics 11: 375-386, 1955).

Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Flag: Critical study for SIDS endpoint
11-OCT-2004 (76) (77) (78)

Type: Sister chromatid exchange assay
System of testing: Chinese hamster ovary cells
Concentration: 0, 16, 50, 160, 500, 1600, 5000 µg/ml
Cytotoxic Concentration: no data about cytotoxicity
Metabolic activation: with and without
Result: negative

Method: other: see freetext, comparable to OECD Guide-line 479
GLP: no

Test substance: other TS: choline chloride, no further data

Result:

Dose in µg/ml	Number of SCEs per cell -MA (% of control)	+MA (% of control)
control	7.4	9.2
16	8.1 (109)	9.2 (100)
50		8.5 (93)
160	7.1 (96)	8.9 (97)
500	7.6 (103)	9.4 (102)
1600	7.3 (100)	8.3 (92)
5000	8.1 (109)	9.3 (101)
positive control	31.4 (424)	41.3 (451)

CONCLUSION
No increase in the incidence of SCEs at any dose level with or without metabolic activation.

Test condition: SYSTEM OF TESTING
- test procedure without MA: cells exposed to the TS for 2 hrs without addition of BrdU; than BrdU was added and exposure continued for 24 hrs; after washing cells incubated in medium containing BrdU and colcemid for 2-3 hrs; cells were then harvested by the mitotic shake-off method, fixed and stained with Hoechst 33258 and Giemsa; 50 second metaphase cells per dose scored for SCEs.
- test procedure with MA: incubation with S9-mix plus TS for 2 hrs without fetal calf serum; then cells incubated in medium containing BrdU and 10% fetal calf serum (no TS) for 26 hrs, with colcemid present the last 2-3 hrs; further preparation see above.
- Metabolic activation (MA) system: S9-mix, liver microsomes prepared from male Sprague-Dawley rats treated with Aroclor1251
- number of chromosomes examined: ca. 1050 per dose level,
- Solvent: supplemented McCoy's 5A medium (culture medium)
- Controls: negative (solvent control) positive control mitomycin C (without MA) or cyclophosphamide (with MA)
- one trial performed
- laboratory: Environmental Health Research & Testing

CRITERIA FOR EVALUATING RESULTS:
- no data

Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions. Restrictions: no repeat trials, no data about cytotoxicity but highest dose similar to dosing in other valid studies.

Flag: Critical study for SIDS endpoint

11-OCT-2004 (78)

Type: Bacterial gene mutation assay
System of testing: exp. design 1) E. coli K12 and exp. design 2) E. coli B
Concentration: 1) 0, 28, or 70 mg/ml for 30 min; 2) 70 mg/ml for 3 h
Cytotoxic Concentration: 28 mg/ml
Metabolic activation: without
Result: negative

Method: other: see freetext
GLP: no
Test substance: other TS: choline chloride, no further data

Remark: No further data available.
Result: EXPERIMENTAL DESIGN 1
- survival 89% (control), 46% (low dose), 39% (high dose)
- 2.2, 2, 3.6 mutants per 10E6 survivors, respectively.

EXPERIMENTAL DESIGN 2
- 0 mutants per 100 survivors; survival 0.5%.

Test condition: EXPERIMENTAL DESIGN 1
- reverse mutation assay using late exponential phase cells
- valine sensitive cells exposed at pH 9 to the TS for 30 min and then plated on valine-containing plates; mutant valine insensitive cells per 10E6 survivors determined.

EXPERIMENTAL DESIGN 2
- forward mutation assay using E. coli B, late exponential phase; cells exposed at pH 9 for 3 h
- auxotrophic mutants determined by the replica-plating technique; no control.

Reliability: (2) valid with restrictions
Meets generally accepted standards, acceptable for assessment.
Valid results only concerning reverse mutation assay performed at pH 9 (for comparison with chlorocholine chloride) without metabolic activation.
Flag: Critical study for SIDS endpoint
13-JUN-2003 (79)

Type: other: gene conversion assay
System of testing: Saccharomyces cerevisiae D4
Concentration: 0, 1.25, 2.5, 5% (0, 12.5, 25, 50 mg/ml)
Cytotoxic Concentration: high dose resulted in 50% survival
Metabolic activation: with and without
Result: negative

Method: other: comparable with OECD Guide-line 481
GLP: no
Test substance: other TS: choline chloride, white crystals, no further data

Result: GENOTOXIC EFFECTS:
- With and without metabolic activation no increase in gene conversion at any dose level.

CONTROLS:
- spontaneous gene conversion in negative controls within the normal range; valid positive controls.

Evaluation:
Under the condition of this study the TS did not cause DNA damage either with or without metabolic activation.
Test condition: SYSTEM OF TESTING
- suspension, 4 h exposure at 30°C; thereafter incubation of yeast plates at 30°C for 3-5 days
- 6 different metabolic activation (MA) systems; S9-mix, liver or lung microsomes prepared from 1) male Sprague-Dawley rats, 2) male ICRFLO mice, 3) male rhesus monkey (all species without pretreatment)

- 2 trials per exp. design
- Solvent: phosphate buffer
- Controls: negative (vehicle control) and positive control ethyl methanesulfonate (-MA) & dimethyl nitrosamine (+MA)
- Cytotoxicity: tested in preliminary studies; cells incubated at 37°C for 4 h with 0.0005, 0.005, 0.05, 0.5, 5% TS in buffer

CRITERIA FOR EVALUATING RESULTS:
considered positive if the TS produced at least a 2-3-fold increase in gene conversions per plate over vehicle control and a dose response to increasing concentrations

Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Flag: Critical study for SIDS endpoint
11-OCT-2004 (74)

Type: Bacterial reverse mutation assay
System of testing: E. coli Sd-4-73
Concentration: see freetext
Cytotoxic Concentration: no data
Metabolic activation: without
Result: negative

Method: other: see freetext
GLP: no
Test substance: other TS: choline chloride, no further data

Result: TS nonmutagenic (no further data).
Test condition: EXPERIMENTAL DESIGN
- Reversion from streptomycin dependence to independence in this strain measured by the paper-disk method;
- mutagenicity manifested as a zone of streptomycin-independent mutant colonies around a filter-paper disk saturated with the TS on the nutrient agar containing streptomycin; inoculum overnight at 36 °C.

Reliability: (4) not assignable
Documentation insufficient for assessment
13-JUN-2003 (80)

Type: Cytogenetic assay
System of testing: Chinese hamster ovary cells
Concentration: no data
Cytotoxic Concentration: no data
Metabolic activation: no data
Result: negative

Method: other: no data
GLP: no data
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
No further data available

13-JUN-2003 (81)

Type: Sister chromatid exchange assay
System of testing: Chinese hamster ovary cells
Concentration: no data

Cytotoxic Concentration: no data
Metabolic activation: no data
Result: negative

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Reliability: (4) not assignable
Secondary literature
No further data available
13-JUN-2003 (81)

5.6 Genetic Toxicity 'in Vivo'

5.7 Carcinogenicity

5.8.1 Toxicity to Fertility

Type: Fertility
Species: rat
Sex: male
Strain: no data
Route of administration: i.p.
Exposure Period: Exp. design 1) 12 days; exp. design 2) 24 days
Frequency of treatment: once daily
Doses: 0 or 25 mg/rat (0 or ca. 80 mg/kg bw/day) in exp. design 1 & 2
Control Group: yes, concurrent vehicle

Method: other: see freetext
Year: 1993
GLP: no data
Test substance: other TS: choline chloride, no further data

Result: The body weight gain as well as the testes weight in treated rats was not altered. Also other organ weights showed no difference to control values (epididymis, liver, kidney, adrenal gland measured).

12 DAYS TREATMENT
- 2 days after exposure epithelial vacuoles observed at later stages; spermatogonia showed pyknotic nuclei; cellular debris in a few tubules was seen; normal architecture reported 5 days after treatment period (reversibility of effects);
- no effects detected in quantitative analysis

24 DAYS TREATMENT
- 2 days after exposure only a few tubules at stage I-IV were damaged but at stage V-VI epithelial vacuolisation was noted; most tubules at stage IX-XIII were damaged: blebbing of Sertoli cell apical cytoplasm, dislodging of pachytene spermatocytes, and inappropriate arrangement of

spermatid bundles;
in earlier stage tubules evidence of late pachytenes degeneration was detected; late pachytenes were highly eosinophilic;
- 5 days after treatment period spermatogonia and spermatocytes were normal but several pachytenes were necrotic; at stage I-IV the population of elongated spermatids seemed slightly decreased;
- after 8 days post treatment: gaps at the expected position of the elongated spermatids detected at stage XIII-XIV;
- at day 12 normal architecture of the germinal epithelium was reported except a few necrotic pachytenes
- quantification revealed significantly depleted pachytenes at post treatment days 2-5 (slight increase on next days, nonsignificant difference compared with control value), while a significant increase of spermatogonia was noted from day 5 onwards; no effects concerning zygotenes;

CONCLUSION
Prolonged i.p. administration of choline may be toxic to male reproduction. However, the effects seem to be reversible.

Test condition:
- Male rats (body weight 300 g) received i.p. applications of the TS for 12 (n=25) or 24 (n=25) consecutive days (one cycle of the seminiferous epithelium takes ca. 12 days);
- control rats (n=10 per treatment period) were injected i.p. with distilled water;
- via the diet all rats ingested ca. 10-13 mg choline/kg bw/day;
- TS treated rats were sacrificed 2, 5, 8, and 12 days after the treatment period (presumably n=5 per post exposure observation period, not documented);
all controls were sacrificed 12 days after the treatment period;
- both testes were weighed and one testis per animal prepared for histopathology;
- quantification of spermatogonia, zygotenes, pachytenes in 10 randomly selected tubules at stage XII for each post exposure observation period.

Reliability: (2) valid with restrictions
Meets generally accepted standards, acceptable for assessment.
Restrictions: only one dose, control group only one post exposure observation period (12 days).

Flag: Critical study for SIDS endpoint

11-OCT-2004 (82)

5.8.2 Developmental Toxicity/Teratogenicity

Species:	mouse	**Sex:**	female
Strain:	NMRI		
Route of administration:	oral feed		
Exposure period:	gestation day 1-18		
Frequency of treatment:	pregnant mice received daily ca. 5 g of the prepared diet		
Duration of test:	18 days		
Doses:	1, 2.5, 5 or 10 % TS in the diet (ca. 1250, 4160, 10800, 20000 mg/kg bw and day)		

Control Group: yes
NOAEL Maternal Toxity: = 1250 mg/kg bw
NOAEL Fetotoxicity : = 1250 mg/kg bw

Method: other: see freetext
GLP: no
Test substance: other TS: choline chloride, no further data

Remark: The study was conducted before test guidelines were established to determine the toxicity of this endpoint. The following differences to the OECD Test Guideline 414 (Prenatal Developmental Toxicity Study) were noted:
- Rat/rabbit are recommended species according to OECD
- Test substance was not applied by oral gavage but by feed every other day
- The number of pregnant animals with implantation sites should be at least 20
- No data on uterine weights are reported nor data on sex distribution of foetuses

It is further noted that the lowest dose of substance application in this study was above the limit dose of 1000 mg/kg bw/day. The other dose groups exceeded this value up to twenty fold.
No malformations are seen at any dose. There were not sufficient pups for a NOAEL

Result: MATERNAL TOXICITY

	median body weight during exposure	average weight gain of dams in g without resorption	with resorption
dose			
1%	40 g	25.2 (n=16)	- (n=0)
2.5%	30 g	16.8 (n=8)	2.7 (n=4)
5%	30 g	12.6 (n=3)	0.2 (n=8)
10%	25 g	- (n=0)	-5.2 (n=7)

Reduced weight gain of mothers also without resorption. No symptoms detected in the low dose group (presumably also related to body weight gain compared with controls; control data not shown).

DEVELOPMENTAL TOXICITY
- resorption of all embryos in the high dose group, in 8 pregnant mice after 5% TS in the diet and in 4 after 2.5%; only implantation sites observable.

Dose	total number of resorptions (number of exposed mothers)	% resorptions/total number of implantations
control	12 (414)	(0.28%)
1%	0 (16)	(0%)
2.5%	39 (12)	(35%)
5%	77 (11)	(69%)
10%	68 (7)	(100%)

Developmental toxicity

average number of foetuses	average foetal weight in g	average foetal length in cm	resorptions (in %) number of living pups

Dose				
control	9.5	1.3	2.2	343 (7.99%) 3918
1%	10.3	1.4	2.4	7 (4.0%) 166
2.5%	5.8	1.2	2.2	4 (3.6%) 69
5%	2.9	0.9	2.0	2 (1.8%) 32
10%	-	-	-	-

Developmental toxicity
- after 1% TS in the diet 2 out of 166 (1.2%) foetuses showed cleft palate (control 40/3918; 1.02%) and 1 fused ribs (control: 6 out of 3918 showed malformation of the ribs)
- fused ribs also in 1 out of 32 foetuses after 5% in the diet
- no effects in other groups

Test condition: PREPARATION OF THE DIET
- aqueous suspension of the TS in Traganth mixed with the ground diet; dried at 80°C for 14-15 hrs.

EXPOSURE
- each pregnant mouse received every 2nd day one piece of the prepared diet (ca. 9.5-11 g, no data about control); average doses calculated for each group
- number of pregnant mice per group: 414 (control, presumably not concurrent but historical), 16 (1% in the diet), 12 (2.5%), 11 (5%), 7 (10%); all mice housed singly
- all pregnant mice sacrificed on gestation day 19; fetuses and uteri examined; no statistical evaluation

Reliability: (2) valid with restrictions
Meets generally accepted standards, acceptable for assessment.

Flag: Critical study for SIDS endpoint

11-NOV-2004 (83)

Species: mouse **Sex:** female
Strain: NMRI
Route of administration: i.p.
Exposure period: gestation day 11-15
Frequency of treatment: once daily
Duration of test: 5 days
Doses: 60 and 160 mg/kg bw/day
Control Group: yes, historical

Method: other
GLP: no
Test substance: other TS: choline chloride, no further data

Remark: The doses applied (60 and 160 mg/kg bw/day) are within a factor of 2-3 (high dose) and 6-8 (low dose) below the acute LD50 i.p. (350 - 500 mg/kg bw) determined in BASF studies with two test substance preparations.
The study was conducted before test guidelines were established to determine the toxicity of this endpoint. The following differences to the OECD Test Guideline 414 (Prenatal Developmental Toxicity Study) were noted:

- Rat/Rabbit are the recommended species according to OECD
- The i.p. route of exposure is not a recommended procedure
- The time window chosen for substance application will detect only a selection of effects on late organogenesis
- The number of animals studied does not correspond to the minimal number of pregnant animals with implantation sites recommended by the guideline (20).

Therefore the results of the study are regarded to be of restricted value.
As the layout of this study will not detect effects on early development starting with the implantation phase, a NOAEL for Developmental Toxicity cannot be derived from this study.

The frequency of cleft palates observed are not dose dependent (4.1% in the low dose group, 1.5% in the high dose group, 1.02% in the historical control, no concurrent control group). The observed cleft palates in the low dose group are therefore not assessed as being substance related.

Result: MATERNAL TOXICITY
- no data presented

DEVELOPMENTAL TOXICITY

	average number of foetuses	average foetal weight in g	average foetal length in cm	resorptions (in %) number of living pups
Dose in mg/kg bw				
control	9.5	1.3	2.2	343 (7.99%) 3918
60	9.8	1.2	2.3	5 (4.8%) 98
160	9.7	1.2	2.2	8 (10.5%) 68

- after 60 mg/kg bw 4 out of 98 (4.1%) foetuses showed cleft palate (control 40/3918; 1.02%) (no statistical evaluation); this effect also observed in 1/68 of the high dose group

Test condition: 10 pregnant mice in the low dose group and 7 in the high dose group received the TS in an aqueous solution.

Reliability: (3) invalid
Significant methodological deficiencies

28-FEB-2005 (84)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Type of experience: other: humans, selection of literature

Remark: Remark on selection of literature

The scientific literature of choline comprises thousands of published studies and reviews due its functions as a precursor for acetylcholine, phospholipids, and the methyl donor betaine and its use as dietary component and pharmaceutical.
Retrieval (at beginning of March 2003) by substance name (Choline) or CAS-No. (62-49-7) in MEDLINE and TOXLINE (the two most relevant medical /toxicological databases) resulted in already 27575 hits, respectively 395 hits. Retrieval for choline chloride (by name or CAS-No. 67-48-1) alone however resulted in zero hits in MEDLINE and 65 hits in TOXLINE suggesting that choline salts were not uncompromisingly encoded in these databases and therefore retrieval by choline chloride alone seems to be not useful. Restricting the retrieval to the definite CAS-No. of choline (62-49-7) and using "human" as qualifier still resulted in 3029 in MEDLINE and TOXLINE.
Therefore with a focus on health and safety issues in the frame of ICCA HPV program comprehensive reviews including those of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes of the Institute of Medicine (2000), Zeisel (2000) and Life Science Research Office (LSRO) / Federation of American Societies for Experimental Biology (FASEB) (1981) and studies cited in these reviews were chosen for this data set.

Flag: Critical study for SIDS endpoint

04-OCT-2004

Type of experience: other: humans, review

Remark: Based on analysis of nutrient metabolism in humans and data on intakes in U.S. population, intakes for each age group - from the first days of life through childhood, sexual maturity, pregnancy, lactation, midlife, and the later years were recommended. The primary criterion used to estimated the adequate intake (AI) for choline is the prevention of liver damage as assessed by measuring serum alanin aminotransferase levels.
The AI for adults is 550 mg/day of choline for men and 425 mg/day for women (during pregnancy 450 mg/day and during lactation 550 mg/day).
Choline is in the diet available as free choline or is bound as esters such as phosphocholine, glycerophosphocholine, sphingomyelin, or phosphatidylcholine. Estimated average choline dietary intake in adults consuming typical U.S or Canadian diet (as free choline and the choline in phosphatidylcholine and other choline esters) is approximately 730 to 1,040 mg/day (7 to 10 mmol/day).
Choline is available as a dietary supplement as choline chloride or choline bitartrate and as lecithin, which usually contains approximately 25 percent phosphatidylcholine or 3 to 4 percent choline by weight. There are no reliable estimates of the frequency of use or amount of these dietary supplements consumed by individuals in the U.S. and Canada.
In the treatment of neurological diseases large doses (5 to 30 g) of choline and phosphatidylcholine have been administered to humans.
The critical adverse effect from high intake of choline is

hypotension, with corroborative evidence on cholinergic side effects (e.g. sweating and diarrhoea) and fishy body odour. The tolerable upper intake level applied for chronic daily use for adults is 3.5 g/day.

Reliability: (4) not assignable

secondary literature, see remark of selection of literature

Flag: Critical study for SIDS endpoint

28-SEP-2004 (85)

Type of experience: other: humans, review

Remark: Review on choline as an essential nutrient for humans.

Reliability: (4) not assignable
secondary literature, see remark fo selection of literature

04-OCT-2004 (86)

Type of experience: other: humans, review

Remark: 16-20 g/day of choline chloride were regarded to approximated the highest tolerable dose. Orally administered choline above this dose is limited by the occurrence of gastrointestinal side effects. In the USA, an adequate intake of 550 mg daily for men and 425 mg daily for women has been determined for choline. The tolerable upper intake level for adults is 3500 mg daily.

Reliability: (4) not assignable
secondary literature

04-OCT-2004 (87)

Type of experience: other: humans, kinetics, excretion

Remark: In a study with four subjects (3 women and one man) receiving long-term total parenteral nutrition on consecutive days, 7, 14, 28, and 56 mmol (8000 mg, highest dose) were intravenously infused over a 12-hour period in each subject.
Plasma free choline at baseline before each application was 5.2+-2.1 nmol/ml (normal value 11.4+-3.7 nmol/ml).
In all 4 subjects an overall increase in plasma choline level was observed during the 4 days of intermittent TS infusion; highest plasma levels were measured in most cases 6 h after start of the infusion and lowest levels after 12 h after the end of infusion period (no detailed quantification available from graphs).
The choline concentration in urine varied on day 1 between 3.1 and 44.2 nmol/ml, increased the next days, and reached 212 nmol/ml in subject 1 and up to 12970 nmol/ml in subject 4; also the quantity of excreted choline increased during the 4 days of intermittent TS infusion, from 0.24-1.3 µmol/kg bw on day 1 to 20 (subject 1) - 123 µmol/kg bw (subject 4) on day 4. Urinary choline output increased with plasma choline concentration.
Different models were checked for suitability of plasma choline pharmacokinetic parameters. For all 4 subjects a two compartment model in which elimination from a central compartment is saturable gave the best fit. This model allowed estimates of different parameters (35-37 measurements per subject).
1) rate of endogenous choline turnover in the central

compartment: 3.08-19 µmol/kg/h
2) clearance of choline: 0.43-5.83 l/kg/h
3) rate constant for elimination at nonsaturating concentrations: 1.8-18 per h
4) first order rate constant for transfer between the central and the peripheral compartment: 1.29-2.9 per h
5) affinity constant for the saturable elimination process: 0.019-0.215 ml/nmol
6) clearance of endogenous choline at plasma concentration before the infusion: 3.26-7.09 nmol/ml
7) volume of distribution of choline in the central compartment: 222-591 ml/kg
8) total apparent volume of distribution of choline: 3.91-11.3 l/kg
9) maximal choline clearance: 1.06-9.92 µmol/kg/h
10) quantity of choline in the central compartment: 1.57-2.22 µmol/kg
11) total quantity of choline: 21.4-40.9 µmol/kg
Preparation of the TS: TS dissolved in water (50% solution) and solution sterilized, analysis of TS in the solution revealed 95% recovery.

Subjects: 3 women and 1 man (50+-13 years old) receiving long-term total parenteral nutrition (TPN) for 9.7+-4.7 years were studied; all had low plasma free choline levels.

Procedure: On day 1 1000 mg TS (7 mmol) was added to the 2 l bag of TPN; on the 2nd day 2000 mg TS (14 mmol) was added, the 3rd day 4000 mg (28 mmol), and the 4th day 8000 mg TS (56 mmol); the 2 l TPN was infused at a rate of 167 ml/h for 12 h, starting at 9 a.m. each day, resulting in infusion rates for the TS of 9.95-12.43 µmol/kg/h on day 1, 19.89-24.87 µmol/kg/h on day 2, 39.79-49.74 µmol/kg/h on day 3 and 79.58-99.48 µmol/kg/h on day 4; blood samples were obtained for baseline values and 0.25, 0.5, 3, 6, 12, 15, 24 h after start of infusion; 24-h-urine was collected on each study day, no urinary data available from subject 2.

Side effects: Only one subject had mild nausea, headache and sweating during infusion of 8000 mg (56 mmol, high dose) on day 4 (infusion rate 97 µmol/kg/h; body weight 48 kg), the choline plasma level was 230 nmol/ml; these effects were reversible 2-3 h after infusion was stopped.

Reliability: (2) valid with restrictions
acceptable study; restriction: parenteral application

Flag: Critical study for SIDS endpoint

04-OCT-2004 (88)

Type of experience: other: humans, nutrition

Remark: Humans ingest substantial amounts of choline and lecithin as part of common foods. Physicians have recently begun administering large doses of these compounds to individuals with neurological diseases. A significant fraction of ingested choline is destroyed by enzymes within gut bacteria, forming trimethylamine (TMA), dimethylamine (DMA) and monomethylamine (MMA). Some of these methylamines are eventually excreted into the urine, presumably after being absorbed and carried to the kidneys via the bloodstream.

The methylamines formed after choline is eaten could be substrates for the formation of nitrosamines, which have marked carcinogenic activity. Twenty-seven millimoles of choline chloride, choline stearate or lecithin were administered to healthy human subjects. It was found that these treatments markedly increased the urinary excretion of TMA, DMA and MMA, with choline chloride having the greatest effect. Rats were treated with 2 mmol/kg body weight of choline chloride or lecithin, and it was found that these treatments significantly increased urinary TMA excretion and did not alter DMA or MMA excretion. Our choline chloride preparation contained no MMA, DMA or TMA; however, it was found that our choline stearate and all the commercially available lecithins tested were contaminated with methylamines. Prior removal of methylamines from our lecithin preparation minimized the effect of oral administration of this compound on methylamine excretion in urine of rats and humans.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (89)

Type of experience: other: humans, nutrition

Remark: In a study it was determined whether acute starvation also depletes choline, as indicated by changes in plasma choline or phosphatidylcholine. Healthy humans (n = 10) fasted for 7 d, ingesting only water and mineral-vitamin supplements. Their mean (+/- SEM) plasma choline concentration was 9.5 +/- 0.5 micromol/L at the start of the study and dropped to 7.8 +/- 0.3 micromol/L after 1 wk of fasting ($P < 0.01$). The plasma phosphatidylcholine concentration did not change significantly (2.2 +/- 0.1 mmol/L at the start of the study and 2.4 +/- 0.2 mmol/L after 1 wk of fasting). Capacity of the liver to secrete lipoproteins was not affected by prolonged fasting. The mean plasma concentration of low-density-lipoprotein cholesterol was 3.3 +/- 0.2 mmol/L (126 +/- 8 mg/dL) at the start of the study and 4.9 +/- 0.5 mmol/L (188 +/- 19 mg/dL) after 1 wk of fasting. Liver damage assessed by serum alanine aminotransferase activity occurred in only 1 of 10 subjects. Prolonged fasting in humans modestly diminished plasma choline, but was not associated with signs of choline deficiency, such as perturbed lipoprotein secretion and liver damage.
In the discussion of the results the authors cited, that fasting plasma choline concentrations in humans vary from 9 to 20 µmol/L, with most subjects having concentrations near 10 µmol/L.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

28-SEP-2004 (90)

Type of experience: other: humans, nutrition

Remark: Healthy male volunteers were hospitalised and fed a semisynthetic diet devoid of choline supplemented with 500 mg/day choline for 1 wk. Subjects were randomly divided into two groups, one that continued to receive choline (control), and the other that received no choline

(deficient) for three additional wk. During the 5th wk of the study all subjects received choline. The semisynthetic diet contained adequate, but no excess, methionine. In the choline-deficient group, plasma choline and phosphatidylcholine concentrations decreased an average of 30% during the 3-wk period when a choline-deficient diet was ingested; plasma and erythrocyte phosphatidylcholine decreased 15%; no such changes occurred in the control group. In the choline-deficient group, serum alanine aminotransferase activity increased steadily from a mean of 0.42 µkat/liter to a mean of 0.62 mkat/liter during the 3-wk period when a choline-deficient diet was ingested; no such change occurred in the control group. Other tests of liver and renal function were unchanged in both groups during the study. Serum cholesterol decreased an average of 15% in the deficient group and did not change in the control group. Healthy humans consuming a choline-deficient diet for 3 wk had depleted stores of choline in tissues and developed signs of incipient liver dysfunction.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (91)

Type of experience: other: humans, nutrition

Remark: Ten healthy adult men were fed a diet low in folate and exogenous methyl groups to study the effects on in vivo methylation capability. The men were housed in a metabolic unit for the entire 108 d of the study. After a 9-d baseline period (Period 1), the men were fed a soy-product-amino acid defined diet for 45 d, which provided 25 micrograms/d of folate for 30 d (Period 2) and, with a folate supplement, 99 micrograms/d for 15 d (Period

3). During Period 2 and Period 3, the low methionine and choline diet was supplemented with methionine for half the subjects to vary the dietary methyl group intake. The periods were then repeated over the next 54 d (Periods 4-6), with a crossover of methionine intakes in Period 5 and Period 6. A 1-g oral dose of nicotinamide was given at the end of each period and methylated urine metabolites determined. Other measures related to in vivo methylation capability included urine creatinine, and plasma and urine carnitine. Even with moderate folate depletion, none of these measures was decreased by low methionine and choline intakes. Plasma methionine concentrations were unchanged throughout. Liver function is not reported.

Reliability: (2) valid with restrictions
acceptable study

04-OCT-2004 (92)

Type of experience: other: humans, nutrition

Remark: In a group of 15 patients receiving home total parenteral nutrition who had low plasma free choline levels (6.3 +/- 0.8 mmol/L), 50% had hepatic steatosis. These patients were given oral lecithin or placebo in a double-blind randomised trial for 6 weeks. Lecithin supplementation led to an increase in plasma free choline of 53.4% +/- 15.4% at 2

weeks (P = 0.04), which continued at 6 weeks. The placebo group had no change in plasma-free choline at 2 weeks, but a significant decrease of 25.4% +/- 7.1% (P = 0.01) at 6 weeks. A significant and progressive decrease in hepatic fat was indicated by increased liver-spleen CT Hounsfield units at 2 and 6 weeks (7.5 +/- 1.7 units, P = 0.02; 13.8 +/- 3.5 units, P = 0.03) in the lecithin supplemental group. Nonsignificant changes were seen in the placebo group.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (93)

Type of experience: other: humans, nutrition

Remark: In 41 subjects (19 male , 22 female) aged 45.1 +/- 24.3 years who have received parenteral nutrition for 5.5 +/- 4.7 years plasma free and phospholipid bound choline levels , serum albumin, ALT and AST were determined. Also determined were the daily volume of intravenous lipid emulsion received by the patients as well as the concentration of free choline and phospholipid bound choline in the lipid emulsion. Plasma free choline was low in 33/41 subjects (mean 71.5 +/- 2.5 nmol/ml, normal 11.4 +/- 3.7). Phospholipid bound choline was normal in 34/41 subjects (mean 2157 +/- 620 nmol/ml, normal 2364 +/- 774). Elevation in ALT and AST were significantly correlated with plasma free choline but not with phospholipid bound choline. No relationship was found between age, parenteral nutrition duration or daily volume of intravenous lipids and plasma free or phospholipid bound choline. The lipid emulsion contained 24 +/- 6 nmol/ml of free choline and 11630 +/- 552 nmol/ml of phospholipid bound choline.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (94)

Type of experience: other: humans, nutrition

Remark: Four patients (1 man, 3 women) aged 50 +/- 13 years who had low plasma-free choline concentrations 4.8 +/- 1.7 (normal, 11.4 +/- 3.7 nmol/mL) were studied. The patients had received TPN for 9.7 +/- 4.7 years. They received parenteral nutrition solutions containing choline chloride (1 to 4 g/d) for 6 weeks. Abdominal computed tomography (CT) was performed at baseline, biweekly during the choline supplementation, and 4 weeks after discontinuation of choline. During choline administration, the plasma-free choline concentration increased into the normal range within 1 week in all four patients and remained at or above the normal range for all 6 weeks, but decreased back to baseline when choline supplementation was discontinued. Hepatic steatosis resolved completely, as estimated by CT. Liver density increased from -14.2 +/- 22.3 Hounsfield units (HU) to 8.4 +/- 10.3 HU at week 2 (P = .002); 9.6 +/- 10.7 HU at week 4 and 13.1 +/- 7.3 HU at week 6, as determined by the liver-spleen CT number difference obtained by the subtraction of the average spleen CT number

(in HU) from the average liver CT number. This improvement continued up to 4 weeks after choline supplementation (13.8 +/- 2.8 HU). Hepatic steatosis was shown to have recurred in one patient after 10 weeks of return to choline-free parenteral nutrition.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

28-SEP-2004 (95)

Type of experience: other: humans, nutrition

Remark: The prevalence of plasma choline deficiency by determining fasting plasma levels of choline among cirrhotic and noncirrhotic malnourished male subjects maintained on regular hospital mixed food or elemental parenteral and enteral formulas was estimated. Plasma choline concentrations (microM, average +/- SD) were as follows: (i) mixed foods, 11.3 +/- 4.3 for cirrhotic (n = 22) and 9.3 +/- 2.4 for noncirrhotic (n = 12) patients; (ii) parenteral formula, 5.3 +/- 1.6 for cirrhotic (n = 5) and 8.6 +/- 5.2 for noncirrhotic (n = 16) subjects; and (iii) enteral formula, 6.1 +/- 1.2 for cirrhotic (n = 5) and 11.7 +/- 1.9 for noncirrhotic (n = 4) subjects. The level for healthy normal subjects eating mixed foods was 12.0 +/- 2.2. The prevalence of plasma choline deficiency, i.e., plasma levels greater than or equal to 2 SD below the normal average, was as follows: parenteral formula, all cirrhotic and 10 of 16 noncirrhotic subjects; enteral formula, all cirrhotic and none of the noncirrhotic subjects. The reversibility of choline deficiency was examined in a longitudinal study of three phases involving 10 patients - 5 with alcoholic cirrhosis (all on enteral formula); 5 noncirrhotic (1 on enteral and 4 on parenteral formula). During phase 1 (3-day equilibration period; ad libitum regular hospital diet), plasma choline levels were within the normal range for all subjects. During phase 2 (2 wk, choline depletion phase, elemental formulas), choline levels were subnormal in all cirrhotic subjects (5.1 +/- 2.0 microM) on enteral formula and all noncirrhotic patients on parenteral formula (5.9 +/- 1.3 microM). During phase 3 (2 wk, choline repletion phase, elemental formula + 6 g choline/day), the levels normalized in all patients (cirrhotic 11.4 +/- 3.1 microM and noncirrhotic 11.9 +/- 3.2 microM). Analyses of abdominal computed tomographic scans and plasma liver chemistries in the cirrhotic subjects during the three phases suggested a correlation between plasma choline deficiency and hepatic steatosis and abnormal liver enzyme levels in some patients.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (96)

Type of experience: other: humans, nutrition

Remark: Two underweight patients with protein-calorie undernutrition caused by chronic gastrointestinal malabsorption were studied on standard hospital diet, on nasoenteral nutrition, and on total parenteral nutrition. The patients were clinically and metabolically evaluated

during a 5-6 day period of mixed food (choline content 750 mol/80 g protein). Then a 5-11 days course of nasoenteral feeding (choline content 75 mol/80 g protein) was initiated , followed by a two weeks choline-free total enteral nutrition. Then daily oral supplement was given for one week consisting of 4 g choline base and other nutraceuticals. Since plasma choline levels were subnormal choline base was increased to 8 g. Clinically, the supplement was well tolerated, and no side effects occurred. Plasma levels of nutrients were compared with analyses of at least 15 fasting normal controls. In both patients, the plasma levels of cysteine, tyrosine, choline, and carnitine decreased only during total parenteral nutrition and increased with their supplementation during total parenteral nutrition. Plasma tyrosine and choline were increased to normal levels; cysteine and carnitine were elevated, but still subnormal with supplementation.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (97)

Type of experience: other: humans, nutrition

Remark: Plasma samples, obtained periodically during total parenteral nutrition therapy, for choline concentration were analysed. Malnourished patients referred to a nutrition support service were prospectively assigned to be treated with daily infusions of amino acids with, and without, supplemental daily infusions of lipid emulsion for a period of 1 wk. After the first week, all subjects received intravenous lipid, and most were offered enteral food supplements. Initial plasma choline concentrations in the 25 malnourished patients were significantly lower than those measured in plasma samples from 23 hospitalised patients known to be eating well (6.5 +/- 0.6 vs 9.7 +/- 0.7 nmol/ml; mean +/- SEM; p less than 0.001). During the first week of TPN therapy, plasma choline concentrations in the lipid-restricted group tended to decrease (from 7.3 +/- 1.0 to 4.7 +/- 0.5 nmol/ml; mean +/- SEM; p less than 0.05), while in the lipid-supplemented group plasma choline tended to increase (from 5.6 +/- 0.5 to 6.2 +/- 0.7 nmol/ml; mean +/- SEM; p less than 0.05). Plasma choline concentration increased during wk 2-4, when all patients were treated with lipid emulsions, and some were offered enteral foods.

Reliability: (2) valid with restrictions
acceptable study

04-OCT-2004 (98)

Type of experience: other: humans, nutrition

Remark: Free plasma choline levels were measured in 15 patients before and after total parenteral nutrition. Free plasma choline levels 2, 4, and 6 weeks after the start of total parenteral nutrition were abnormally low and significantly below pre-total parenteral nutrition levels. Pre-total parenteral nutrition and SGPT level was normal(18 +/- 2 IU/l); activity rose at 2 weeks (61 +/- 15) and fell to normal at 4 (36 +/- 8) and 6 weeks (23 +/- 3). SGOT,

alkaline phosphatase, and total bilirubin levels were normal pre-total parenteral nutrition and did not change significantly.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (99)

Type of experience: other: humans, nutrition

Remark: A prospective study was performed in clinically malnourished patients in which liver function was tested during a 4-week period of total parenteral nutrition (TPN). The purpose was to determine if concomitant intravenous lipid administration would reduce liver function abnormalities noted to occur frequently in patients receiving TPN. Twenty-five patients were randomly assigned to receive either daily infusions of 200 cc of a 20% lipid emulsion with TPN or TPN without lipid for the first week. In the subsequent 3 weeks all patients received daily intravenous lipid. The early lipid treatment group received 0.7 g lipid/kg BW/day and approximately 280 mg of choline/day from the lecithin emulsifier throughout the entire study period. Liver function tests were performed twice in the first week, then weekly thereafter. There were significant (p less than 0.05) elevations in liver function tests in the early lipid treatment group (for aspartate aminotransferase in weeks 1, 2, and 3, and lactic acid dehydrogenase in weeks 2 and 3). Alkaline phosphatase activity was elevated at weeks 2, 3, and 4 for the lipid-treatment group and at week 1 for the lipid-restricted group. The two groups had a similar elevation in gamma-glutamyltransferase (GGT)activity. Analysis of covariance demonstrated that the overall duration of TPN, and not the presence or absence of intravenous lipid, was significantly related to the elevations in both alkaline phosphatase and gamma-glutamyltransferase (GGT) levels. In contrast, the early intravenous administration of lipid was significantly related to the increase in aspartate aminotransferase levels. The peak increase in AST was noted at day 7 in the lipid-administration group.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (100)

Type of experience: other: humans, nutrition, review

Remark: Healthy humans fed with a choline-deficient diet for three weeks developed biochemical changes consistent with choline deficiency. These included diminished plasma acholine and phosphatidylcholine concentrations as well as diminished erythrocyte membrane phosphatidylcholine concentrations. Serum alanine transaminase (ALT) activity increased significantly during choline deficiency. Malnourished humans, in whom stores of choline, methionine, and folate have been depleted, appear to need more dietary choline than healthy adult subjects did. The liver is the primary

site for endogenous synthesis of choline. Alcoholics with liver cirrhosis have diminished plasma choline concentration and fatty liver, which resolves when patients are supplemented with choline. Humans treated with parenteral nutrition required 1 - 1.7 mmol of choline-containing phospholipid daily during the first week of parenteral nutrition therapy to maintain plasma choline levels. Decreased plasma choline concentrations in parenteral nutrition patients were reported at the same time when liver dysfunction was detected. Conditions that enhance hepatic triglyceride synthesis (such as carbohydrate loading) increase the requirement for choline. Thus, treatment of malnourished patients with high-calorie parenteral nutrition solutions at the time of depleted choline stores might enhance the likelihood of hepatic dysfunction.

Reliability: (4) not assignable
secondary literature; see remark of selection of literature

Flag: Critical study for SIDS endpoint

04-OCT-2004 (101)

Type of experience: other: humans, nutrition, review

Remark: The bioavailability of dietary choline depends on intestinal absorption: it is absorbed via mediated transport from the lumen of the duodenum, jejunum, and ileum. Some choline is metabolised by gut bacteria to betaine and methylamines before absorption can occur. Dietary phosphatidylcholine is broken down by phospholipases A1, A2, and B present in pancreatic secretions and intestinal mucosal cells. The resulting free choline enters the portal circulation of the liver.

Reliability: (4) not assignable
secondary literature; see remark on selection of literature

Flag: Critical study for SIDS endpoint

04-OCT-2004 (102)

Type of experience: other: humans, acute toxicity, oral

Remark: The suicide of a young man with the plant growth regulator Cycocel (R) (chlorocholine chloride and choline chloride, no quantities given) is reported. After alcohol consumption the man ingested approx. 4 l of Cycocel with vomiting and coma and finally death. Autopsy showed brain swelling, general hemorrhagia, red-violet swelling of the mucous membranes of the upper gastrointestinal tract, fatty degeneration of the myocard and liver cells. Blood alcohol level was 1,9 g/kg.

Reliability: (2) valid with restrictions
acceptable study, restrictions

04-OCT-2004 (103)

Type of experience: other: humans, acute toxicity, oral

Remark: A case of a 21-year-old woman who had developed mild hepatotoxicity while receiving choline magnesium trisalicylate therapy is described. She presented with fever and mild hepatic enzyme elevations before choline magnesium trisalicylate therapy was instituted. Liver function tests (LFT) returned to normal within five days of

hospitalisation but she continued to develop daily fevers. Blood, urine, and throat cultures were negative. An acute viral illness or reactivation of systemic lupus erythematosus were the suspected diagnoses. Choline magnesium trisalicylate (1500 mg bid) was then administered in an effort to control her fever, and was successful. After three days of choline magenesium trisalicylate therapy her LFT values began to rise. They continued to rise for five more days before choline magnesium trisalicylate hepatotoxicity was suspected. Choline magnesium trisalicylate was discontinued after eight days and the patient's LFT quickly returned to normal. The source of fever was never identified, although infection with cytomegalovirus was considered the most likely cause. However, it is possible that the viral illness may have predisposed her to salicylate hepatotoxicity (a subject already described elsewhere), the authors suggested.

Reliability: (2) valid with restrictions
acceptable study

10-NOV-2004 (104)

Type of experience: other: humans, acute toxicity, oral

Remark: Case report of severe hypersensitivity hepatitis with striking tissue and peripheral eosinophilia after ingestion of choline magnesium trisalicylate. A 66-year-old retired nurse took a 3-day course of choline magnesium trisalicylate, 750 mg bid) for treatment of osteoarthritis. Three days later, her eyes turned yellow, her stools lightened, her urine darkened, and she complained of anorexia, nausea, and occasional vomiting, but denied rash of fever. Laboratory examination revealed the following: normal electrolytes, AST 1257 IU/L, ALT 774 IU/L, total bilirubin 14.3 mg/dl, direct bilirubin 7.0 mg/dl, LDH 460 IU/L, alkaline phosphatase 542 IU/L, GGT 949 IU/L, prothrombin time 12.6 s, and white blood count 11,500 (53 % granulocytes, 15 % lymphocytes, 32 % eosinophils). The total eosinophil count was 1725 at 2 days after admission. During initial week of hospitalisation, her liver function tests continued to rise, with transaminases peaking on the 4th hospital day at AST 4420 IU/L, ALT 1800 IU/L, and total bilirubin 21.3 mg/dl. Skinny needle liver biopsy revealed dramatic lobular hepatitis with marked ballooning of hepatocytes and prominent regenerative features. The lobular infiltrate consisted primarily of lymphocytes, with

many scattered eosinophils. There was focal hepatocytic dropout and collapse of the adjacent reticulin framework, but no bridging necrosis or fibrosis was identified. No granulomas or viral inclusion were identified. The patient was discharged after 3 weeks of hospitalisation, when her symptoms subsided and transaminases had returned to near normal levels. A reaction to salicylates (a subject already described elsewhere) was suggested by the authors.

Reliability: (2) valid with restrictions
acceptable study

04-OCT-2004 (105)

Type of experience: other: humans, acute toxicity, oral

Remark: Changes in serum choline, glucose, insulin, cortisol, prolactin, cholesterol, and triglyceride levels resulting from ingestion of low- or high-choline meals in 16 normal human subjects were measured. After consumption of a single meal containing 3 g choline chloride, serum choline rose by 86 %($p<0.01$), attaining peak values after 30 min. When the same subjects ate a meal containing an equivalent amount of choline in the form of lecithin, serum choline levels rose by 33 % after 30 min., and continued to rise for at least 12 hr., to 265 % over control values ($p<0.001$). Serum choline concentrations were related to the amount of choline in the diet; they did not vary significantly during 24-hr. periods when the subjects consumed a low-choline diet for two consecutive days, but rose substantially ($p<0.01$) after each high-choline meal. Serum glucose, insulin, cortisol, and prolactin levels were not significantly modified by choline or lecithin ingestion. Consumption of high choline diet significantly elevated serum triglyceride levels ($p<0.01$) and depressed serum cholesterol ($p<0.01$).

Reliability: (2) valid with restrictions
acceptable study

04-OCT-2004 (106)

Type of experience: other: humans, skin

Remark: A 21-Day Cumulative Irritation study on 25 subjects with self-perceived sensitive skin was conducted. The materials evaluated were 0.5 % choline chloride aqueous solution, a soap bar containing 5 % choline chloride and a liquid body soap containing 5 % choline chloride. The soap bar and liquid body soap formulas (both 1.0 % w/v aqueous solutions) and 0.5 % choline chloride aqueous solution and vehicle control (water) were evaluated. The positive control was 0.75 % (w/v) sodium lauryl sulphate. The controls were the respective choline chloride-free samples: water, choline -free soap bar, and choline-free liquid body soap. Test samples were applied to the back of volunteer subjects under semi-occlusive patch conditions. Twenty-four hours after application, the patches were removed, the sites evaluated for signs of irritation, and identical patches applied to the same sites. This procedure was repeated daily for a period of 21 consecutive days, although patches applied on a Friday were not removed until the next Monday. Statistical analysis of the cumulative irritancy demonstrated no significant differences between the samples containing choline chloride and their respective choline chloride free controls.

Reliability: (4) not assignable
only secondary literature

Flag: Critical study for SIDS endpoint

08-MAR-2004 (107)

Type of experience: other: humans, skin

Remark: A Human Repeated Insult Patch Test was done on two hundred two subjects. The test concentration was 0.5 % (w/v) choline chloride aqueous solution during the induction phase and 0.2 % (w/v) aqueous solution during the challenge phase. The vehicle (distilled water) was used as the

control. In addition, 0.1 % (w/v) sodium lauryl sulphate was used as an internal control to assess subject compliance. The patch conditions were occlusive patch. During the induction phase, the test material was patched for 24 hours on the back of the volunteer subjects; 48 h post-application sites were evaluated and identical patches applied. Sites patched on Friday were evaluated the following Monday, though. A rest period of two weeks followed the induction phase. During the challenge phase, the test material was patched for 24 h to previously unexposed sites and the sites were evaluated 48- and 72-h post application. The results of the study showed no evidence of dermal sensitisation reactions elicited by choline chloride.

Reliability: (4) not assignable
only secondary literature

Flag: Critical study for SIDS endpoint

04-OCT-2004 (108)

Type of experience: other: humans, skin

Remark: A 23-year-old woman, employed in a garden centre for six months, developed an acute dermatitis of the hands, arms and face. Patch testing was positive for Cycocel (R) (chlormequat chloride and choline chloride) (10 % in pet.) and choline chloride (1 % in water and in pet.), whereas 1 % chlormequat chloride was negative. Control tests with the two substances in 10 patients were negative.

Reliability: (2) valid with restrictions
basic data given, restrictions

08-MAR-2004 (109)

Type of experience: other: humans, repeated dose toxicity, oral, review

Remark: The small amounts of free choline normally present in the diet can be rapidly absorbed by the intestine. However, if choline is ingested in large amounts in supplements, a large part is converted by bacteria in the intestine to trimethylamine and trimethylamine oxide. Considerable amounts of these products are absorbed and excreted in sweat and urine. This not only produces an objectionable body odour, but also limits the amount of choline that can be utilised from ingestion.
Total choline in the food may amount to 500-900 mg/d. Both exogenous and endogenous choline are transported to the liver by portal circulation. The liver metabolises choline and releases phosphatidylcholine and very small amounts of choline to the plasma. The fasting level of plasma choline in man has been found to vary from 7 - 22 µM/l.

Biological side effects have been demonstrated only with large amounts. 16-20 g/d of choline is suggested to be the highest tolerable dose. 30 g may have produced cardiac arrhythmia.
The peripheral cholinergic effects of large doses of choline are nausea, vomiting, salivation, sweating, and anorexia.

Reliability: (4) not assignable
secondary literature, see remark of selection of literature

Flag: Critical study for SIDS endpoint
04-OCT-2004 (110) (111)

Type of experience: other: humans, repeated dose toxicity, oral

Remark: Twenty-three patients with rheumatoid arthritis were given choline magnesium trisalicylate (CMT) (Trilisate; Adcock-Ingram) in a dose of 1.5 g (3 tablets) twice daily and were followed up for 6 weeks. Nineteen patients completed the study and the data obtained were subjected to statistical analysis. There was a statistically significant improvement in the indices of inflammation. Seven patients developed tinnitus, which resolved on reduction of the dose to 1 g (2 tablets) twice daily. Four patients developed pruritus and minor gastro-intestinal side-effects were present in 3 patients, but all these side-effects were transient and no change in therapy was necessary.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint
04-OCT-2004 (112)

Type of experience: other: humans, repeated dose toxicity, oral

Remark: Patients with Alzheimer´s disease were given 2 weeks treatment with oral choline chloride in suspension at a dose of 5 g daily, then a further 2 weeks at 10 g daily (which is equivalent to 3.75 g (36 mmol) or 7.5 g (72 mmol) of choline alone). Patients general tolerated choline chloride 5 g/day well. Daily blood-pressure recording revealed no hypotension and there were no reports of nausea or diarrhoea and no other evidence of peripheral cholinergic stimulation. At 10 g daily some patients experienced nausea and diarrhoea, and in these patients there was a small fall in blood-pressure. There was no testable improvement in cognitive function. The dose of 7.5 g of choline was regarded as a LOAEL by the Standing Committee on the Scientific Evaluation of Dietary Reference Intake, 2000.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint
04-OCT-2004 (113) (85)

Type of experience: other: humans, repeated dose toxicity, oral

Remark: In a clinical study five patients with tardive dyskinesia and eight patients with Huntington´s disease where placed whenever possible on a placebo-choline-placebo-choline regimen in which patients receive up to 20 g of choline chloride per day. All of the patients experienced a significant reduction in the frequency of their choreiform movements during the second 4-week choline period compared to the immediately preceding placebo period. Adverse effects were not reported.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint
04-OCT-2004 (114)

Type of experience: other: humans, repeated dose toxicity, oral

Remark: Nine male patients with chronic schizophrenia were given choline chloride dissolved in distilled water to a concentration of 0.5 g/ml. All patients were started with an initial dose of 1 g/day of choline chloride. The dose was gradually increased by 1 g four times a day every 2 or 3 days, until patients received a total of 20 g/day. they were maintained at this dose for 3 or 4 weeks, then switched to placebo. No significant change in clinical ratings of schizophrenics when treated with choline was seen. With placebo a change to a significant depression was noted. No adverse effects were reported.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (115)

Type of experience: other: humans, repeated dose toxicity, oral

Remark: Pharmacologic doses of choline were given to patients with tardive dyskinesia in an attempt to suppress involuntary facial movements. Twenty patients with stable buccal-lingual-masticatory movements took oral doses of choline for two weeks according to a double-blind crossover protocol (150 mg per kilogram per day during the first week and 200 mg per kilogram per day during the second week). Half the patients received choline, and the other half placebo, for two weeks; these schedules were reversed after 10-day interval during neither choline nor placebo was given. Plasma choline levels rose from 12.4 +/- 1.0 to 33.5 +/- 2.5 nmol per milliliter (mean +/- S.E.M.; P less than 0.001) during this period. Choreic movements decreased in nine patients, worsened in one and were unchanged in 10. No serious side effects were encountered in any subject during the course of the study. Two patients were more withdrawn than usual and possibly depressed during choline treatment. Three patients experienced symptoms of mild cholinergic toxicity, including lacrimation, blurred vision, anorexia and diarrhoea, while taking 200 mg of choline per kilogram per day. All the effects were dose related and subsided when the dosage was reduced.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (116)

Type of experience: other: humans, repeated dose toxicity, oral

Remark: A 39-year-old man with classical buccolingual-masticatory dyskinesia was given choline chloride. Only slight changes in abnormal movements were noted in the patient on choline therapy until a dose of 16 g was reached after eight days of treatment. At this point, abnormal movements were decreased markedly. No adverse effects were reported.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

14-JUL-2003 (117)

Type of experience: other: humans, repeated dose toxicity, oral

Remark: Report of two cases of depression associated with oral choline. A 29-year-old man treated with tardive dyskinesia received oral choline beginning at 3 g a day, increasing to 9 g over 1.5 weeks. His symptoms developed parallel to the increased dose until, at 9 g, he became highly agitated, paranoid, and even more severely depressed. Choline was withdrawn and restarted 2 weeks later. When choline was increased to 9 g per day, he again developed depression. In a second case of 57-year-old woman with tardive dyskinesia at choline doses increasing to 9 g over to weeks weakness and depressive symptoms developed. After drug withdrawal the symptoms remitted over 7-8 days.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (118)

Type of experience: other: humans, repeated dose toxicity, oral

Remark: Fourteen patients with a variety of ataxic disorders were given choline chloride, double blind for six weeks, in an attempt to improve gait and manual dexterity. For the first three weeks the dose was 4 g/day followed by 150 mg/kg/day for the second three week period. One patient withdrew before receiving the active drug, twelve patients showed no functional improvement, but one achieved greater mobility; his response, which was dose dependent, ceased when choline was stopped and was reproducible. Side effects of choline included nausea and diarrhoea on the higher dose. The characteristic "bad fish" body odour was noted in five patients. Blood pressure and weight was unchanged.

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

14-JUL-2003 (119)

Type of experience: other: humans, repeated dose toxicity, oral

Remark: Oral choline and its natural dietary source, lecithin, was administered to 5 men with mild to severe tardive dyskinesia in a nonblind trial. Both choline and lecithin increased serum choline levels and improved abnormal movements in all patients. As adverse effects mild and transient Parkinsonian signs (bradykinesia, tremor, and rigidity) was observed in high doses (12.7 g/day of choline as a chloride).

Reliability: (2) valid with restrictions
acceptable study

Flag: Critical study for SIDS endpoint

04-OCT-2004 (120)

Type of experience: other: humans, lactation

Remark: The major choline-containing compounds of human milk (unesterified choline, phosphatidylcholine, sphingomyelin) were measured in samples obtained from mothers of full-term infants. Unesterified choline concentrations were highest (greater than 600 nmol/ml) during the first week of

lactation, but thereafter remained relatively constant at 70-200 nmol/ml. There was no difference among foremilk, middle milk and hind milk, nor was there a diurnal pattern of variation in unesterified choline concentrations. Milk phosphatidylcholine and sphingomyelin concentrations remained relatively constant throughout lactation (100-200 nmol/ml). Hind milk always contained more of these phospholipids than did foremilk or middle milk. There was no consistent diurnal pattern of variation in milk concentrations of phosphatidylcholine or sphingomyelin. Milk contained no phospholipase activity capable of forming free choline from phosphatidylcholine or sphingomyelin. Bovine milk contained approximately the same concentrations of choline, phosphatidylcholine and sphingomyelin as did human milk from mothers more than 15 d postpartum. The same was true of "humanized" infant formulas made from cows' milk. Soy protein-based formulas had much more unesterified choline (up to 650 nmol/ml) and much less sphingomyelin than did mature human milk.

Reliability: (2) valid with restrictions
acceptable study

28-FEB-2005 (121)

Type of experience: other: humans, placenta

Remark: Microsomes from human, mouse and rat placenta were found to contain enzymatic activity which methylates the phospholipids phosphatidylethanolamine (PE), phosphatidyl-N-monomethylethanolamine (PMME) and phosphatidyl-N,N-dimethylethanolamine (PDME) to form phosphatidylcholine (PCh) with 3H-methyl-S-adenosyl-l-methionine as the methyl donor. The three labelled reaction products were isolated by solvent extraction and separated on thin-layer chromatography (TLC) plates. The endogenous methyltransferase activity was low, indicating that the methylation pathway is quantitatively not important for the synthesis of free choline to meet the fetal needs. The distribution of 3H-methyl among PMME, PDME and PCh revealed fairly even labelling of all products when analysed by TLC. Addition of authentic PE, PMME and PDME to a level of approximately 2.5 mM stimulated the incorporation of 3H-methyl into the total lipid-soluble fraction with all three substrates, but was most pronounced with PMME. Present observations suggest that all three methylation steps were catalysed by one enzyme with a pH optimum of 9.0 in a reaction that does not require Mg++. During pregnancy in humans large amounts of choline are delivered to the foetus through the placenta.

Reliability: (2) valid with restrictions
acceptable study

04-OCT-2004 (122) (123)

5.11 Additional Remarks

Type: Biochemical or cellular interactions

Result: 1) The concentration of the TS required for 50% inhibtion was 4 mM (rat plasma; cholinesterase), 4,5 mM (human plasma, cholinesterase), 4,0 mM (rat erythrocytes, acetylcholinesterase).
2) Significant decrease ($p<0.005$) compared with control value (80% of control).

Test condition: 1) In in vitro studies the influence of the TS on the activity of acetylcholinesterase in rat erythrocytes and on the activity of cholinesterase in human or rat plasma was tested.
2) Effects on the activity of cholinesterase in the plasma was measured in rats (n=8) 20 min after i.p. injection of 175 mg/kg bw TS.

Test substance: choline chloride, no further data

19-FEB-2003 (124)

Type: Toxicokinetics

Remark: Choline is absorbed from the diet. After large doses free choline is not fully absorbed, and intestinal bacteria metaboize choline to trimethylamine. This substance results in a strong odor of decaying fish to the feces. So lecithin is the preferred oral vehicle for administration of choline. Lecithin is hydrolysed by the intestinal mucosa to glycerophosphoryl choline, which passes to the liver to liberate choline or passes to the peripheral tissue via the intestinal lymphatics.

Reliability: (4) not assignable
Secondary literature
No further data available

20-FEB-2003 (31)

Type: other: anticancerogenic effects

Result: The TS was not effective in inhibiting the development of liver tumors or the corresponding lung metastases.

Test condition: The effect of feeding of choline chloride (1% in the diet) on liver tumor promotion by phenobarbital (PhB) or 1,1 bis(p-chlorophenyl)-2,2,2-trichlorethane (DDT) was studied in rats receiving an initiating dose of 200 mg/kg bw diethylnitrosamine.

Test substance: choline chloride, no further data

24-FEB-2003 (70)

Type: other: effects on neurons & transmitter systems

Remark: Prenatal treatment with TS (dams received via gavage 300 mg/kg bw on gestation day 12-17) resulted in an increased size of NGF (nerve growth factor) receptor positive neurons in the basal forebrain of male and female rats on postnatal day (PD) 30; this effect lasted longer in males than in females (studied on postnatal day 90). Increased levels of NGF protein in hippocampus on PD 20.

Test substance: choline chloride, no further data

21-FEB-2003 (125)

Type: other: effects on neurons & transmitter systems

Remark: Ca. 30 min after i.p. injection of 100 or 120 mg/kg TS (n=6 rats) acetylcholine concentration in striatal dialysates (CNS) increased significantly for 30-45 min. Similar results were presented for dialysate samples from cerebellum and lateral ventricle. Acetylcholine release due to application of choline chloride was potentiated by atropine.

Test substance: choline chloride, no further data

21-FEB-2003 (126)

Type: other: effects on neurons & transmitter systems

Result: TS treatment increased significantly body weight measured on post natal day 70; TS treatment enhanced visuospatial memory tested on postnatal day 80-145; in immunocytochemistry on postnatal day 200 the somata of P75 neurotrophin receptor-immunoreative neurons were significantly larger; this morphological alteration might contribute to improvement in spatial memory.
Similar results were detected in rats treated only prenatally on gestation days 12-17.

Test condition: Pre- and postnatal treatment with choline chloride: Pregnant rats received 3-5 d after conception 300 mg/kg bw per day TS via the drinking water; pups cross-fostered to dams receiving no TS; on postnatal days 1-24 pups once daily s.c. injected with 250 mg/kg bw TS; 20 males and 20 females treated; gonadectomy performed on postnatal day 60; concurrent treatment controls.

Test substance: choline chloride, no further data

14-APR-2003 (127)

Type: other: effects on neurons & transmitter systems

Remark: Prenatal supplementation of rats with choline improves spatial (Williams et al., 2000) and temporal (Meck & Williams, 1997) memory in offspring.
In an electrophysiological study (Montoya et al., 2000) pregnant rats received via the diet and the drinking water 0 (deficient), 180 (control) or 640 mg/kg bw/day (supplemented) TS on gestation day 12-17, followed by standard diet (180 mg/kg bw/day TS) in pre- and postnatal period (for offspring also after weaning).
After postnatal day 30 hippocampal slices were prepared (only male rats) and electrophysiological recordings performed. In TS supplemented rats a significant potentiation of population EPSP slopes (EPSP: excitatory postsynaptic potential) was observed after carbachol treatment.
Conclusion: improved memory function may be mediated by changes in the organization of the hippocampal cholinergic system.

Test substance: choline chloride, no further data

21-FEB-2003 (128) (129) (130)

Type: other: effects on neurons & transmitter systems in neonatal rats

Result: No difference in body weight gain of pregnant rats between treatment group and control; no effects on the number of delivered pups per litter, pup weight at birth, and survival of pups (1st 36 h postnatal); histopathology: no effects in the brain detected but glycogen deposits in pups of TS treated dams; no effects on brain choline or acetylcholine concentration; liver choline concentration significantly increased; phospholipid analysis of the pup brain: significantly increased amount of sphingomyelin and phosphatidic acid, but total phospholipids per g brain significantly decreased.

Test condition: Pregnant Sprague-Dawley rats received the normal diet (control; n=6) or a diet supplemented with 0.8% TS (ca. 500 mg/kg bw/day; n=12); exposure started on gestation day 6 and lasted up to postnatal day 15; neurochemical and phospholipid analysis on newborn pups (n=6 per dose); histopathology performed on 3 newborn pups (only brain and liver).

Test substance: choline chloride, no further data

25-FEB-2003 (131)

Type: other: enhanced survival in leukemic mice

Result: No effect on body weight gain. Supplemented mice showed a significantly prolonged survival.

Test condition: Spontaneously leukemic AK mice (100% mortality expected by 12-13 months of age) were fed an unsupplemented diet or a diet containing 1% TS plus 2% D,L-methionine; 50 female mice per group; exposure started 1 day after weaning.

Test substance: choline chloride, no further data

25-FEB-2003 (132)

Type: other: prediction of clastogenic activity

Remark: Using a computer automated structure evaluation (CASE) method the authors calculated a DNA damaging activity for the TS in the SCE assay and a marginal clastogenic activity in the cytogenetic test in CHO cells. The results published in Galloway et al. (1985; see section 5.5) are presented in this study as positive results (SCE- and cytogenetic assay).

Test substance: choline chloride, no further data

24-FEB-2003 (133)

Type: other: protection against hyperammonemia

Result: 100% mortality in controls was reduced to ca. 20% mortality in TS treated mice.

Test condition: Male Swiss mice received i.p. 222 mg/kg bw TS and 15 min later i.p. 12 mmol/kg ammonium acetate (n=15). 10 Controls received only i.p. 12 mmol/kg ammonium acetate.

Test substance: choline chloride, no further data

20-FEB-2003 (134)

Type: other: toxicity and teratogenicity in chicken embryo

Result: No teratogenic effects in the developing chicken embryo was found. The highest dose tested was 25 mg/egg. The LD50 was 11.1 mg/egg.

Test condition: Eggs were injected through the air cells on the 4th day of incubation; injection volume 100 µl, vehicle water; minimum 5 dose levels, 20 eggs per dose; vehicle control; skeletal and visceral defects investigated; statistical analysis.

Test substance: choline chloride, no further data

25-FEB-2003 (135)

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