FOREWORD INTRODCUTION

Mucochloric acid
CAS: 87-56-9

SIDS Initial Assessment Report

For

SIAM 17

Arona, Italy, 11 -14 November 2003

1.	**Chemical Name:**	Mucochloric acid
2.	**CAS Number:**	87-56-9
3.	**Sponsor Country:**	Germany Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Contact person: Prof. Dr. Ulrich Schlottmann Postfach 12 06 29 D-53048 Bonn-Bad Godesberg
4.	**Shared Partnership with:**	BASF AG, Germany; OXON Italia S.p.A., Italy.
5.	**Roles/Responsibilities of the Partners:**	
•	Name of industry sponsor /consortium	BASF AG, Germany Contact person: Dr. Hubert Lendle, D-67056 Ludwigshafen GUP/CL – Z 570
•	Process used	see next page
6.	**Sponsorship History**	
•	How was the chemical or category brought into the OECD HPV Chemicals Programme ?	by ICCA-Initiative
7.	**Review Process Prior to the SIAM:**	last literature search (update): 15 May 2002 (Ecotoxicology): databases CA, biosis; searchprofile CAS-No. and special search terms 27 June 2003 (Toxicology): databases medline, toxline; search-profile CAS-No. and special search terms
8.	**Quality check process:**	As basis for the SIDS-Dossier the IUCLID was used. All data have been checked and validated by BUA.
9.	**Date of Submission:**	August 12, 2003
10.	**Date of last Update:**	

11. Comments:

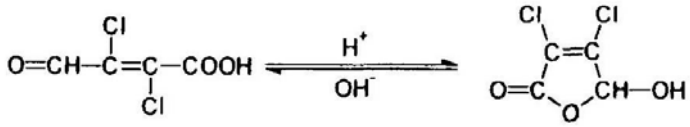
OECD/ICCA - The BUA* Peer Review Process

Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA guidelines/instructions for the OECD/ICCA peer review process and includes:

- a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
- Review of data and assessment of the quality of data
- Review of data evaluation
- Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications
- Review of key study description according robust summaries requirements; completeness and correctness is checked against original reports/publications (if original reports are missing: reliability (4), i.e. reliability not assignable)
- Review of validity of structure-activity relationships
- Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)
- In case of data gaps, review of testing plan or rationale for not testing

* BUA (GDCh-Beratergremium für Altstoffe): Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	87-56-9
Chemical Name	Mucochloric acid
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

There are no reliable experimental data on the toxicokinetic behavior of mucochloric acid (MCA) *in vivo* available. From the results of acute toxicity studies, it is very likely that MCA itself or its metabolites are systemically available after oral exposure. *In vitro*, MCA reacted with N-acetylcysteine, cysteine and glutathione (GSH).

The acute toxicity (LD_{50}) of MCA was between 300 and 400 mg/kg bw in rats after oral exposure and >200 mg/kg bw (highest tested dose) in rabbits after dermal exposure. The LC_{50} after 4-hour inhalation exposure of rats was >5.1 mg/l (highest tested concentration). Clinical signs included atonia and ataxia after oral exposure, preening, dyspnoea and salivation during inhalation, and skin irritation after dermal exposure.
MCA is corrosive to the rabbit skin and eye. A guinea pig sensitization test was negative, but limited experience from occupational exposure in humans indicates a skin sensitizing potential of MCA.

There is limited data on repeated dose toxicity available, indicating that irritant/corrosive effects at the site of first contact are the main effects to be expected after repeated exposure. In pregnant rats, no systemic target organ has been identified after oral exposure from day 6 to 19 p.c. (LOAEL: 30 mg/kg bw/day, based on reduced food consumption and body weight gain together with minor clinical symptoms (ptyalism) and whitish foci in the stomach interpreted as local effects due to the corrosive properties of MCA; NOAEL: 5 mg/kg bw/day). No target organ was identified in mice after dietary exposure to 7 mg/kg bw/day for 18 months (only one dose tested). Because of the limited exposure potential and the availability of reliable, though limited, data on repeat dose toxicity, no further animal testing is warranted.

In vitro, MCA is a direct acting mutagen and clastogen in mammalian and bacterial cells, and forms exocyclic DNA adducts. *In vivo*, mucochloric acid caused a slight, but statistically significant increase in the incidence of total nuclear anomalies (including micronuclei, pyknotic nuclei and karyorrhectic nuclei) in the duodenum of mice after a single oral exposure to 60.8 and 79.4 mg/kg bw. MCA induced micronuclei in one animal out of ten per dose group in the duodenum of mice after single oral doses (38.9, 60.8, and 79.4 mg/kg bw). Based on the available *in vitro* and *in vivo* data, it can be concluded that MCA has a genotoxic potential.

Because of its corrosive properties, and the very limited exposure potential, animal tests with MCA for its effects on fertility were not performed. In an oral developmental study performed in accordance with OECD TG 414 in rats, the NOAEL for maternal toxicity was 5 mg/kg bw/day. The NOAEL for developmental toxicity was 60 mg/kg bw/day, which was the highest dose level applied. There were no signs of developmental toxicity or teratogenicity.
MCA did not induce aberrant crypt foci or intestinal tumors when given in drinking water at dose levels of 0.45 and 0.9 mg/ml over 6 weeks to rats or at dose levels of 0.18 and 0.35 mg/ml over 4 weeks with subsequent 12-weeks recovery to mice, respectively. The available data for MCA are not sufficient to judge its carcinogenicity. Given the available data for genotoxicity there are, however, concerns with regard to this endpoint.

Environment

The solubility of MCA in water is approximately 27 g/l (pH 2.2) at 20 °C and the vapor pressure is 0.00139 hPa at 25 °C. A Henry's law constant of $8.7*10^{-4}$ $Pa*m^3*mol^{-1}$ can be calculated and the partition coefficient log K_{ow} was measured as 0.697 at 25 °C. The acid-base constant (pKa) is 4.20 at 25 °C.

The distribution modeling (Mackay fugacity model level I), indicates water to be the almost exclusive target compartment. The substance has no considerable potential for bioaccumulation (log K_{ow} = 0.697). It cannot be considered inherently biodegradable according to OECD guidelines, but is partially biodegradable after an appropriate adaptation. From the structure of MCA hydrolysis is not expected. Photodegradation is to be expected under environmental conditions with an estimated half-life of 21.4 h. An estimated K_{oc} value of 1 indicates that mucochloric acid does not tend to adsorb to soil. However, as the structure of the molecule is dependant on pH, the K_{oc} may vary significantly with pH.
Aquatic effects data are available for three trophic levels (fish: LC_{50} (96 h) = 123 mg/l; crustacea: EC_{50} (48 h) = 13 mg/l; algae: E_rC_{50} (72 h) = 65 mg/l, E_bC_{50} (72 h) = 62 mg/l). A $PNEC_{aqua}$ of 13 µg/l was calculated from the available data using an assessment factor of 1000 according to the EU Technical Guidance Document.

Exposure

In the EU there are only two known producers of MCA. The annual production volume in the EU is in the range of 1000 - 5000 tons. There is no information on imported volumes. In Eastern Europe, there is one producer in Slovakia, who produces MCA only for captive use. In China there are two known producers.
MCA was used in the 1970s and 1980s, as a gelatin hardener in the photographic industry and as an intermediate in the pharmaceutical production. Since 1990 MCA has only been used as the starting material for the production of two herbicidal substances: Chloridazon and Norflurazon. Production of MCA and processing to Chloridazon takes place in the EU in closed systems at the same site. For the production of Norflurazon, also in closed systems, MCA is transported under controlled conditions in so-called big bags to a single site in the USA and filled into the reactor via docking the big bags to it. During production, transport and processing of MCA personal protective equipment is used to minimize any workers exposure to the substance. At the production and processing sites in the sponsor country, workplaces are regularly monitored.
MCA is not emitted into the atmosphere; due to its low vapor pressure only traces of MCA are expected in the off-gases, which are held back in the scrubber liquids. There is no emission into the aquatic environment during production and processing of MCA at the sponsor company; any remaining aqueous solution is oxidized or incinerated. Solid wastes, which contain less than 100 ppm of MCA, are disposed of by incineration.
There is no evidence of MCA in the finished products, i.e. herbicide formulations (analytical detection limit: 5 ppm). Because MCA is only used as an intermediate in closed systems and transported under strictly controlled conditions, there is very limited exposure potential from these sources in the sponsor country, both for humans and the environment.
In certain countries, and independent of its production and processing by the chemical industry, MCA was found in surface waters (µg/l range) resulting from effluents from chlorine bleaching processes and in drinking water (ng/l range) as a chlorination disinfectant byproduct from the reaction of chlorine with humic acids.

RECOMMENDATION

The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health:
The chemical possesses properties indicating a hazard for human health (corrosivity, genotoxicity, potential carcinogenicity), but controls for occupational exposure are in place in OECD countries.
In view of concerns that the chemical may be a genotoxic carcinogen, there is a recommendation for sharing the toxicological and exposure data with regulatory agencies responsible for drinking water, because traces of MCA can occur in drinking water as a disinfection by-product. Based on this, countries may want to consider toxicokinetic/metabolism studies, and, if then indicated, further studies relating to the carcinogenicity endpoint.

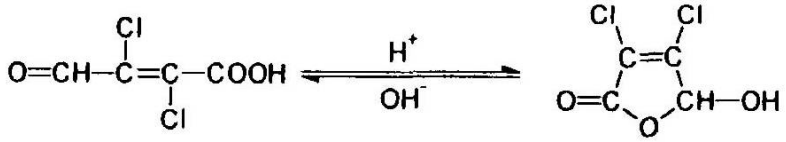
Environment:
The chemical possesses properties indicating a hazard for the environment. Based on data presented by the Sponsor country, exposure to the environment is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

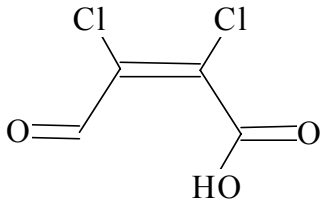
SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 87-56-9
IUPAC Name: 2,3-Dichloro-4-oxo-2-butenoic acid,
Molecular Formula: $C_4H_2Cl_2O_3$
Structural Formula:





000087-56-9 2-Butenoic acid, 2,3-dichloro-4-oxo-, (Z)-

Molecular Weight: 168.96 g/mol
Synonyms: Acrylic acid, 2,3-dichloro-3-formyl
Aldehydrodichloromaleic acid
2-Butenoic acid, 2,3-dichloro-4-oxo-, (Z)- (9CI)
α,β-Dichloro-β-formylacrylic acid
2,3-Dichlor-3-formyl-acrylsaeure
2,3-Dichloro-3-formyl acrylic acid
2,3-Dichloromaleinaldehydsaeure
2,3-Dichloromaleic aldehyde acid
3,4-Dichloro-2-hydroxycrotonolactone
3,4-Dichloro-2-hydroxycrotonolactonic acid
3,4-Dichloro-5-hydroxy-2[5H]furanone
Dichloromalealdehydric acid
Dichloromaleic aldehyde acid
2,3-Dichloro-4-oxo-2-butenoic acid
Malealdehydic acid, dichloro- (7CI, 8CI)
Mucochloric acid
Mucochloric acid (6CI)
Mucochlorsaeure

1.2 Purity/Impurities/Additives

≥ 93% w/w (H_2O <1%; HCl <1%)

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Comments / References
Physical state	Solid	
Color	Colourless-yellowish	
Odor	Characteristic-pungent	
Melting point	124 - 127 °C	Hommel, 1992
Bulk Density	750 - 800 kg/m^3 950 kg/m^3	DIN 53 468 / BASF, 1970 BASF, 1999a
Vapor pressure	0.00139 hPa	Calculated: MPBPWIN v.1.40, US EPA (2000); modified Grain method / BASF, 2002a
Water solubility	27 g/l	at 20 °C / Hommel, 1992
pH	2.2	at 24 g/l / BASF, 1999a
Dissociation constant pKa	4.20	at 25 °C / Serjeant and Dempsey, 1979
Partition coefficient n-octanol/water (log value)	0.697	at 25 °C / BASF, 1988a
Henry's law constant	$8.7*10^{-4}$ Pa*m^3*mol^{-1}	calculated based on mol mass, vapor pressure and solubility / BASF, 2002b; BASF, 2002c
Flash point	100 °C > 100 °C > 127 °C	Hommel, 1992 DIN 51 758 / BASF, 1976 BASF, 1999a
Auto flammability	Not self heating	Method VDI 2263 part 1, 1.4.1BASF, 1976
Flammability	Not highly flammable	Method VDI 2263 part 1, 1.2; BASF, 1976
Explosive properties	Not explosive	Method comparable to 92/69/EEC, A 14 1; BASF, 1976
Oxidizing properties	No oxidizing properties	BASF, 1999b
Hazardous reactions	Exothermic reaction with alkalis	BASF, 1999a

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

In the EU there are only two known producers of mucochloric acid (MCA), one in Germany and the other in Italy. The annual production volume in the European Union (EU) is in the range between 1000 and 5000 tons. There is no known import of MCA into the EU. Outside the EU there are known producers in Slovakia and China. According to information from the Slovakian Contact Point, the Slovakian producer produces MCA only for captive use. A Chinese company specifies on its internet page that it produces 2000 tons of MCA per year (http://www.czxt.com/gsjj_e.htm). From another known Chinese producer (http://www.pumeng.com/mucochloricacid.htm) no data on production volumes are available. These Asian producers are not involved in the OECD HPV Chemicals program, and the reliability of the aforementioned information can therefore not be evaluated. There is no information about further production sites available.

In the EU, MCA was sold to several customers in the 1970s and 1980s. It is not known for what purpose MCA was used except for its use as a gelatin hardener in the photographic industry and as an intermediate in the pharmaceutical production.

Since 1990, MCA is only used by the European producers as the starting material for the production of two herbicidal substances: Chloridazon and Norflurazon. Both are active ingredients for a series of formulated plant protection products. The production of Chloridazon takes place in the EU at the sites in Germany and Italy where also MCA is produced. The production takes place in a closed system. MCA for Norflurazon production is sold only to one customer in the USA. Norflurazon is produced in the USA also in a closed system.

Thus, based on the information available to the Sponsor country, MCA can be regarded as an intermediate with controlled transport to only one processing site.

The type of consignment for MCA transport is polypropylene bags, 1377 lbs per bag, the so-called "big bags". MCA is trucked in full container loads from the manufacturing site in Italy to the nearest seaport and then sent by ocean freight from Italy to the USA. The annual volume shipped there is around 1 million lbs (ca. 450 tons). There the big bags are trucked again from the port of entry to the site, where the bags (one per pallet) are off-loaded with a forklift. The MCA bags are moved from the warehouse to the manufacturing plant by a forklift, suspended above the reaction kettle and discharged into the reactor via docking the big bags to it. The entry point of the reactor is equipped with a dust collector. Thus, considering the low vapor pressure of MCA and the fact that the big bags are not handled by manpower but using technical equipment (fork lift) the system can also be regarded as a nearly closed system. In addition, the operators are required to wear full protective clothing. The emptied big bags are disposed of by land filling. Given that MCA is very water-soluble and is predicted to partition predominantly to water, is not readily biodegraded and is unlikely to bind strongly to soil (based on its K_{oc}), migration of MCA into the environment via landfill leachate should be considered an additional exposure pathway.

The product is labelled as follows:

Land transport:	ADR Class 8	corrosive materials
	Number/letter	65b
	Kemler number	80
	UN number	1759
	Label	8
	Designation of goods	1759, corrosive, solid. mucochloric acid.
Maritime transport:	IMDG Class	8
	Page	8151
	UN number	1759
	Packing group	II
	EMS number	8-15
	MFAG	760
	Correct technical name: corrosive, solid. mucochloric acid.	

Internet search on trading of MCA revealed that it is offered, e.g. in the USA, in China, and the Ukraine (http://www.wegochem.com/DyePigmentIntermediates.htm, http://www.ammetals.com/chem-organic.htm, http://www.dpsoe.sumy.ua/eng/price.shtml; http://www.dsl-intl.com/Other.htm). MCA is offered as an organic intermediate e.g. for dye, pigments, pesticide and pharmaceutical production. To which extent MCA is sold and used is not known to the sponsor company. MCA is

also marketed in small quantities as a laboratory chemical for professional users in chemical laboratories. Since the amounts are small and the personnel involved is generally well-trained and accustomed to handling hazardous chemicals, the exposure is very limited.

MCA is not contained in the Danish, Swedish and Swiss product registers (Danish Product Register, 2002; Swedish Product Register, 2002; Swiss Product Register, 2001).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Releases into the environment may occur during production and processing of mucochloric acid. A non-quantifiable exposure of the terrestrial compartment may occur from possible residual contents of mucochloric acid in the subsequent products that are used as plant protection products. The production and processing of MCA at the sponsor company takes place in a closed system in the presence of water. The solid material is separated. The remaining aqueous solution is oxidized respectively incinerated. MCA is not emitted into the atmosphere; due to its low vapor pressure only traces of MCA are expected in the off-gases, which are held back in the scrubber liquids.

In the course of the two-step synthesis of Chloridazon and Norflurazon MCA is consumed due to its reactivity, except for trace amounts remaining in the active ingredient, the process waters and the waste. In the finished product, no MCA is usually detectable at the analytical detection limit of < 5 ppm MCA. The solid wastes coming from the solvent recycling operations contain less than 100 ppm of MCA. These wastes are disposed of by incineration.

Thus, no significant releases of MCA into the environment during production or processing at the sponsor company or use of the distributed products are identified. As pointed out above MCA can be regarded as an intermediate with controlled transport to one site.

Independent of its commercial life cycle, MCA can be present in the aqueous environment as a result of chlorine bleaching and chlorine-disinfection of drinking water. Among other chlorinated organic compounds, MCA can be formed by reaction of chlorine with natural organic matter (NOM), particularly humic acids. In a determination of chlorinated furanones and hydroxy-furanones in pulp bleaching liquor, in chlorine-treated natural humic water and in Finnish chlorine-treated drinking water, Kronberg and Franzén (1993) detected MCA in nearly all extracts. In a sample of chlorination-stage bleaching liquor derived from pine craft pulp, which was prebleached with oxygen they detected 67 µg MCA/l. In natural humic water, MCA concentrations of about 2.5 µg/l were found after chlorination. Maximal concentrations of MCA found in chlorinated drinking waters were around 10 – 60 ng/l. In a more recent study Smeds et al. (1999) found levels up to 12 ng/l in several of 35 investigated Finnish and one Russian drinking water samples. For comparison, the related compound 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone), generally known as MX, was found in the same range, i.e. at levels of 0.4 - 80 ng/l (Hemming et al., 1986; Suzuki and Nakaniski, 1990; Kronberg and Franzén, 1993; Wright et al., 2002). A detailed elaboration of this inadvertent formation of MCA is beyond the scope of this SIAR. A comprehensive review on disinfectants and disinfectant by-products was published by the WHO (2000). With regard to halogenated hydroxyfuranones, no data on levels of MCA are given (see also 3.2).

2.2.2 Photodegradation

Rapid degradation in air is to be expected based on the calculated reaction of MCA with the hydroxyl radical which results in a photodegradation half-life of $t_{1/2}$ = 21.4 hours (BASF, 2003).

2.2.3 Stability in Water

From the structure of MCA hydrolysis is not to be expected. The pH dependant equilibrium between the 2 isomers as such does not represent a hydrolysis. The hydrolytic cleavage of the chlorine atoms attached to the olefinic double bond is not possible because of lacking reactivity of these chlorine atoms.

At low pH, it is likely that mucochloric acid exists primarily as 3,4-dichloro-5-hydroxy-5H-furan-2-one, but under neutral or alkaline conditions the open chain form is likely to predominate.

2.2.4 Transport between Environmental Compartments

The distribution modelling using Mackay, Level I, which is calculated with the values of mol mass, vapor pressure, solubility and partition coefficient, indicates water to be the almost exclusive (99.9 %) target compartment for the undissociated molecule (BASF, 2002b; 2002c). It has to be considered, that at very low concentrations of MCA expected in the environment, the substance is mostly present as anion (i.e. deprotonated). As anions are neither subjects to volatilization nor to adsorption, the hydrosphere is also the target compartment for the deprotonated molecule.

A Henry's law constant of $8.7*10^{-4}$ Pa*m³/mol was calculated for mucochloric acid based on the above described values for water solubility and vapor pressure. Thus, mucochloric acid is not volatile from aqueous solution.

A K_{OC} value of 1 estimated with the model PCKOWIN 1.6 indicates that the substance does not tend to adsorb to soil. However, as the structure of the molecule is dependant on pH, the K_{OC} may vary significantly with pH.

2.2.5 Biodegradation

The available biodegradation tests are, on the whole, sufficient to characterize the biodegradability of MCA, although the documentations of the test reports are generally limited. For an assessment of the biodegradability of MCA also non-assignable studies are considered. In a 28-day respirometric test, the substance was slightly biologically degraded only in two out of 10 test vessels indicating that the inoculum, which was reported as adapted activate sludge, was not sufficiently adapted. Because of the considerable divergences in the test vessels the test was considered not reliable (BASF, 1981a). In a subsequent respirometer test with an obviously better adapted inoculum, an average degradation of about 75 % (based on BOD*100/COD) or about 67 % (based on DOC elimination) occurred (BASF, 1981b). In a number of Zahn-Wellens tests, which were run considerably longer than 28 days, elimination of the test substance started after a lag phase of several days, regardless of whether adapted or non-adapted activated sludge was used. Since MCA has a very low volatility from water (Henrys Law Constant: $8.7*10^{-4}$ Pa*m^3*mol^{-1}), most of the elimination observed can be considered as biodegradation. Two tests conducted with adapted inoculum are considered reliable based on the available documentation. At the end of these tests, DOC elimination was 40 - 50 % (40 days) (BASF, 1981c) and 70 - 80 % (45 days) (BASF, 1981d), respectively. In other tests, which cannot be evaluated appropriately due to limited documentation, DOC elimination was 80 - 90 % (54 days) (BASF, 1981e), 80 - 90 % (36 days) (BASF, 1981f), 90 - 100 % (35 days) (BASF, 1981g), and 59 % (87 days) (BASF, 1982), respectively. After 28 days, elimination of the test substance was always considerably below 70 % in all these tests. In one test (BASF, 1981g), the elimination was 62 % after 28 days. However, in this test, as in most other such tests, the removal of the test substance did not follow a gradual elimination pattern due to partial and partly considerable DOC increases. This was probably caused by a bacteriostatic effect of the test substance resulting in a temporary disintegration of bacteria flocks. In a BOD_5 test with non-adapted inoculum no biodegradation was noted (BOD/COD < 0.004) (BASF, 1981h).

Conclusions: MCA cannot be considered as inherently biodegradable according to OECD Guidelines, but is partially biodegradable/eliminable.

2.2.6 Bioaccumulation

No experimental data on bioaccumulation are available. The log K_{OW} of 0.697 (BASF, 1988a) indicates a low potential for bioaccumulation.

2.2.7 Other Information on Environmental Fate

No data are available.

2.3 Human Exposure

2.3.1 Occupational Exposure

The two European producers produce MCA in a closed process by the reaction of furfural with chlorine in the presence of water. The solid product is separated; and the remaining aqueous solution is oxidized or incinerated.

In Germany the production of MCA and the subsequent production of Chloridazon take place at a single production site in a closed system. The first step of the production, the chlorination of furfural takes place in the presence of water in a circulation reactor that is covered on the inside with ceramic tile. At the deepest site of the reactor chlorine, furfural, the inert gas nitrogen and water are added, which are processed in an exothermic reaction. The native HCl is exhausted with nitrogen. The continuous conversion of furfural into MCA is supervised by collecting samples at the outlet of the reactor. The personnel have to wear protective clothing, including an Auer 3S mask with respective filter and long-armed neoprene gloves. The second step of the production is the crystallization of MCA. The MCA-suspension draining from the reactor outlet is step wisely cooled down in a crystallization cascade, resulting in crystalline MCA. In case of necessary cleaning processes in the MCA crystallization cascade, the skilled and trained workmen wear protective clothing consisting of a chemical suit with fresh air supply and rubber boots. The crystalline MCA is separated from the extraction liquor by centrifugation in a closed centrifuge system. In case of necessary cleaning of the centrifuge, the workmen wear protective clothing consisting of a chemical suit with fresh air supply and rubber boots. From the centrifuge the solid MCA – slurry is transferred into a closed system (slurry container), from where it is dosed directly into the dichlorpyridazon-cascade to make chloridazon.

Exposure of workers during production is controlled (*cf.* also the above paragraph). Generally, the production units are inspected and repaired annually but also in shorter intervals if necessary. When the production site is opened e.g. for repair or cleaning, appropriate protective measures are applied. Work on the opened system is done only by authorized staff wearing protection suits with admission of fresh air and rubber boots. (see above). Only well trained workers are involved in maintenance of the system. Regular instructions will ensure work safety. These safety instructions e.g. for cleaning of the reactor, are documented and kept centrally in the control room of the production site.

At regular intervals the production unit is surveyed with “check-lists” for any necessary measures (repair, cleaning). Check-lists are available, for instance, for filling and discharge of the reactor content, and for procedures carried out to ensure the safe handling of MCA, HCl and furfural.

In the sponsor country the workers of the MCA producing unit are annually examined by a company medical officer. The results of these examinations are documented. Personal air sampler measurements were taken and examined at regular intervals in accordance with the German technical rules for hazardous substances (TRGS 402).
Single components that were regularly measured included HCl, sulfuric acid, aniline, and substituted benzenes. The values were always in compliance with the limit values. With regard to the educt HCl of the MCA production the measured mean 8 hour value of all measurements performed between 1981 and 2002 was 0.97 mg/m³ (the 90% percentile being 1.7 mg/mg³ and the 70 % percentile being 0.95 mg/m³). The limit value according to German and EU maximum work place concentration for HCl is 8 mg/m³. Moreover, it should be taken into account that the method used until 2001 (direct indication Draeger sampling tubes for HCl, flow 16 ml/min, 480 min sampling time) had a detection limit of 1.3 ppm (= 1.9 mg/m³). So the measured values were in the range of the detection limit. The new method introduced in 2002, a validated ion chromatographic method (IC Anion/BASF, flow 1.16 l/min, 120 min sampling time) with a detection limit of 0.022 mg/m³ revealed a value of less than 0.013 mg/m³. Comparing the old method with new current method the values measured could be reduced to 1.2 % and even with the new method the value was in the range of the detection limit of this method. Taking into account that the reaction of HCl and furfural to MCA is performed in a closed system and that the reaction product MCA is of comparatively low volatility, it can be assumed that the MCA concentration would also be below 0.013 mg/m³.

In Italy, MCA is also produced in a closed system. MCA is stocked in sealed polypropylene bags in a site warehouse or in the processing plant. MCA is transported from the production site in Italy to the customer in the USA in polypropylene bags, 1377 lbs per bag ("big bags"). MCA is trucked in full container loads from the manufacturing site in Italy to the nearest seaport and then sent by ocean freight from Italy to the USA. The annual volume shipped there is around 1 million lbs (ca. 450 tons). There the big bags are trucked again from the port of entry to the site, where the bags (one per pallet) are off-loaded with a forklift. The MCA bags are moved from the warehouse to the manufacturing plant by a forklift, suspended above the reaction kettle and discharged into the reactor via docking the big bags to it. The entry point of the reactor is equipped with a dust collector. Thus, considering the low vapor pressure of MCA and the fact that the big bags are not handled by manpower but using technical equipment (fork lift) the system can also be regarded as a nearly closed system. In addition, the operators are required to wear full protective clothing consisting of rubber suits, neoprene gloves, safety glasses, hard hat and full face respirator equipment with dust and acid filters. The emptied big bags are disposed of by land filling.

In the USA, the potential exposure issues surrounding MCA were addressed during the Hazard and Operability Study (HazOp) for Norflurazon. Personal protection equipment to protect personnel during the process is recommended. Emptied bulk bags can contain residual MCA, therefore a detailed procedure addressing transport of the bags along with their incineration is available.

Workplace monitoring data on mucochloric acid were not available for the Italian, Slovakian and the U.S. sites.

There is no evidence of MCA residues in the finished plant protection products (analytical detection limit: 5 ppm MCA).

In conclusion MCA as produced and used by the two EU producers is an isolated intermediate with a limited potential for exposure. This is based on the fact that it (i) is produced and processed in closed systems, (ii) transported under controlled conditions to only one site of closed processing, (iii) not used or contained in any consumer products and (iv) not present in the form of residues in formulated plant protection products. Thus, no prolonged occupational exposure is to be expected. At most, acute exposure is possible in the case of an accident, for which special safety and protection measures apply because of the corrosive properties of MCA.

2.3.2 Consumer Exposure

There is no direct consumer exposure to MCA because it is not used as such in consumer products and there is no evidence of MCA residues in the finished plant protection products (analytical detection limit: 5 ppm MCA).

Exposure via drinking water can in principle occur if chlorine is used for drinking water disinfection under certain circumstances (pH, presence of humic acids). MCA levels in the ng/l range between 10 and 60 ng/l were found in chlorinated drinking waters (Kronberg and Franzén, 1993). A detailed elaboration of this inadvertent formation of MCA during the chlorination of drinking water is beyond the scope of this SIAR.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

No reliable studies are available on the *in vivo* toxicokinetics of MCA. Based on the toxic effect observed in the acute studies after oral exposure the systemic availability of MCA can be assumed (see **Error! Reference source not found.**).

In vitro MCA reacted with N-acetylcysteine, cysteine and glutathione (LaLonde and Xie, 1992; LaLonde and Xie, 1993; LaLonde et al., 1993; LaLonde et al., 1994). The oxidation of glutathione resulted in conversion of MCA into a carbon-centered free radical (LaLonde et al., 1994).

Based on the available data for MCA and MX it is probable that not MCA itself but metabolites will gain systemic availability. While a large amount of MCA may be detoxified and excreted rapidly metabolites of MCA could be systemically available and biologically active.

Conclusion

There are no reliable experimental data on the toxicokinetic behavior of mucochloric acid (MCA) *in vivo* available. From the results of acute toxicity studies, it is, very likely that MCA itself or its metabolites are systemically available after oral exposure. *In vitro*, MCA reacted with N-acetylcysteine, cysteine and glutathione (GSH).

3.1.2 Acute Toxicity

Of the available acute toxicity studies those meeting generally accepted scientific standards and providing sufficient detail were selected as key studies, and are listed below (Table 2).

Table 2: Acute toxicity of MCA in experimental animals (key studies)

Route	Species[a]	Value	Type	Purity of test substance / Remarks	Reference
Oral	Rat (m/f; in-house breeding; n=10)	300 mg/kg bw	LD_{50}	Technical grade	BASF, 1964a
Oral	Rat (Schmitt-Fischer; n=5-10)	360 mg/kg bw	LD_{50}	Purity ≥ 90 %, neutralized with NaOH	BASF, 1961
Oral	Rat (m/f; Schmitt-Fischer; n=5-10)	400 mg/kg bw	LD_{50}	Purity ≥ 90 %	BASF, 1960a
Inhalation (4 hours / dust aerosol)	Rat (m/f; Sprague-Dawley; n=10)	>5.1 mg/l (measured concentration)	LC_{50}	Technical grade, purity >98 %	BASF, 1980a
Dermal (24 hours, occlusive)	Rabbit (m/f; n=5)	>200 mg/kg bw (the only dose applied)	LD_{50}	Technical grade	BASF, 1977

[a] Data on sex, strain and number (n) per dose in parentheses if available

Inhalation

In a dynamic inhalation test with 4-hour head-nose only exposure, no mortality was observed in rats during exposure or 14-day post-exposure periods. The LC_{50} was >5.1 mg/l. Clinical signs of toxicity included escape attempts, preening, dyspnoea and salivation during exposure. No symptoms were observed 13 days after exposure. Relative body weight gain was significantly reduced in males after 7 and 14 days and slightly reduced in females after 7 days (BASF, 1980a).

Dermal

Administration of a 50% aqueous solution of MCA to the shaved dorsal and side areas of rabbits did not cause any deaths or systemic symptoms of intoxication during and up to 72 hours after exposure. One animal showed slight erythema at the site of application after 96 hours (BASF, 1977).

Oral

After oral application of various specifications of MCA to rats, the LD_{50} was consistently between 300 and 400 mg/kg bw. The toxicity of neutralized MCA was similar to that of the free acid indicating that the toxic effects are substance-inherent and not due to the acidic properties of MCA. Atonia and ataxia were observed as clinical symptoms of toxicity (BASF, 1960a; 1961; 1964a). Gross pathology after the 7-day post-exposure period showed no effects (BASF, 1964a).

Conclusion

The acute toxicity (LD_{50}) of MCA was between 300 and 400 mg/kg bw in rats after oral exposure and >200 mg/kg bw (highest tested dose) in rabbits after dermal exposure. The LC_{50} after 4-hour inhalation exposure of rats was >5.1 mg/l (highest tested concentration). Clinical signs included atonia and ataxia after oral exposure, preening, dyspnoea and salivation during inhalation, and skin irritation after dermal exposure.

3.1.3 Irritation

Skin Irritation

In a test performed according to test guidelines of the US Department of Transportation (Paragraph 173.1200, Federal Register), technical grade MCA was corrosive to rabbit skin (0.5 mg/animal; 2 animals; mean scores after 4 hours, 1, 2, 8 days for erythema: 3.5, 4, 4, 4; for edema: 3, 3, 3, 1.5) after occlusive application for 4 hours (BASF, 1980b). In tests with another exposure scheme (1, 5, 15 minutes and 20 hours), both the pure substance (BASF, 1961) and a highest purity grade specification (BASF, 1964b) were slightly irritating after 15 minutes exposure, but also corrosive after 20 hours exposure. Similar effects resulted if the pure substance was applied after neutralization (BASF, 1961). In all these tests, the substance was applied in 30 - 80% solutions with water. The comparably low irritating effect observed in the acute dermal study (200 mg/kg bw; 50% in water; see above) was probably due to lower concentration per treated skin area.

Conclusion

On 4 to 20 hours exposure, MCA is corrosive to rabbit skin, regardless of the specification or pH value. After very short exposure, the pure substance is only slightly irritating.

Eye Irritation

Today, data on eye irritation are usually not assessed for skin corrosive substances.

For MCA several older studies are available which were performed in accordance with the principles of the Draize test. Different specifications of MCA were corrosive to the eyes of rabbits at observation periods of 8 or 14 days (BASF, 1960b; 1964b). With the pure substance applied after neutralization (pH 6) slight opacity was observed which was reversible after 14 days (BASF, 1961).

Conclusion

MCA is corrosive to the rabbit eye.

3.1.4 Sensitisation

Studies in Animals

In an open epicutaneous skin painting test with guinea pigs (10 treated vs. 3 control animals), neither technical nor highest purity grade MCA showed any sensitizing potential. Twelve hours after challenge of pretreated animals no differences in skin reactions were observed as compared to control animals, which were tested for primary irritation only (BASF, 1964c).

Studies in Humans

Handbook data indicate that MCA may be sensitizing in humans (Patty, 1967). This is based on unpublished data from industry and no further information is provided.

Conclusion

A guinea pig sensitization test that was not conducted according to current guidelines was negative. Limited experience from occupational exposure indicates a skin sensitizing potential of MCA.

3.1.5 Repeated Dose Toxicity

There are no repeated dose toxicity studies available that were performed in accordance with current guidelines and/or standards.

Oral

Some reported subacute and subchronic studies were discounted because they were inadequately documented, used small numbers of animals and/or addressed only few parameters, such as sensory reflexes or activity of cholinesterase (Mashkina and Bathisina, 1971). Limited information on subacute effects of MCA is available from the study on prenatal developmental toxicity in rats described below (BASF, 2001a). Except for one incidental occurrence there were no deaths in all dose groups (5, 30 or 60 mg/kg bw/day by gavage on days 6 to 19 post coitum; administered as a 0.16, 1 or 2 % preparation in olive oil, respectively). The no-observed adverse effect level (NOAEL) for pregnant rats was 5 mg/kg bw/day; the LOAEL was 30 mg/kg bw/day, based on reduced food consumption and body weight gain together with minor clinical symptoms (ptyalism) and whitish foci in the stomach interpreted as local effects due to the corrosive properties of MCA. However, a number of examinations requested in repeat dose guideline studies were not performed. No increase in mortality and no specific effects or target organs were reported from a limited 18-month carcinogenicity study in two different hybrid mouse strains (see below: NCI, 1968; Innes et al., 1969). However, only one dose level was applied (7 mg MCA/kg bw per day in the diet) and clinical examinations were limited. No serum chemistry or hematology examinations were performed.

Conclusion

There are limited data on repeated dose toxicity available, indicating that irritant/corrosive effects at the site of first contact are the main effects to be expected after repeated exposure. In pregnant rats, no systemic target organ has been identified after oral exposure from day 6 to 19 p.c. (LOAEL: 30 mg/kg bw/day, based on reduced food consumption and body weight gain together with minor clinical symptoms (ptyalism) and whitish foci in the stomach interpreted as local effects due to the corrosive properties of MCA; NOAEL: 5 mg/kg bw/day). No target organ was identified either in mice after exposure to 7 mg/kg bw/day in the diet for 18 months (only one dose tested).

MCA is a corrosive substance used mainly in closed systems as a chemical intermediate. Transport of the isolated material is controlled and is limited to a few sites. Exposure is controlled in occupational settings and is negligible for consumers.

Because of the limited exposure potential and the availability of reliable, though limited, data on repeat dose toxicity, no further animal testing is warranted.

3.1.6 Mutagenicity

In vitro Studies

The available *in vitro* studies that are considered key studies are summarized in Table 3.

Table 3: Overview of *in vitro* genotoxicity studies on MCA (key studies)

End-point (species)	Protocol	with[a]	without	Results Remarks	Purity of MCA	Reference
Gene mutation; bacteria						
Reverse mutation (*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538)	Ames test, plate incorporation: 1.25-5000 µg/plate (+ S-9 mix); 0.077-5000 µg/plate (- S-9)	-	+	a) With S-9 mix: all strains negative; cytotoxicity ≥100 µg/plate b) Without S-9 mix: positive for TA98, TA100, TA1535 (cytotox. ≥20 µg/plate); negative for TA1537, TA1538 (cytotox. ≥40 µg/plate)	99%	BASF, 1981i
Reverse mutation (*S. typhimurium* TA 100)	Ames test, plate incorporation: 1.25-500 µg/plate (+ S-9 mix); 1.25-10 µg/plate (- S-9)	+	+	a) With S-9 mix: positive at 1.25 and 2.5 µg/plate (cytotox. >2.5 µg/plate) b) Without S-9 mix: positive at 1.25-10 µg/plate (no cytotoxicity)	Technical grade, pure (twice crystallized; ca. 99.9 %	BASF, 1985
Reverse mutation (*S. typhimurium* TA 100)	Ames test, plate incorporation: Comparative study on several chlorohydroxyfuranones including MCA	+	+	Stronger effects without metabolic activation; without S-9 mix positive at 0.8845 - 5.07 µg/plate; Net TA-100 revertants/nmol MCA: without metabolic activation 60; with metabolic activation 5.0	99 % (Source: Sigma-Aldrich)	Ishiguro et al., 1988

Table 3 (cont.): Overview of *in vitro* genotoxicity studies on MCA (key studies)

End-point (species)	Protocol	with[a]	without	Results Remarks	Purity of MCA	Reference
DNA damage; bacteria						
DNA damage (*E. coli* uvrB/recA lac$^+$ vs. uvr$^+$/rec$^+$ lac$^-$)	Differential DNA repair assay: 0.04-10 µg/ml (+/- S-9 mix or BSA[b])	(+)	+	a) With S-9 mix: "almost complete loss" of genotoxic activity (no data); with BSA: rel. survival rate (%) ca. 24, 62, 96, 108 at 0, 5, 10, 15 mg BSA/ml, resp. b) Without S-9 mix: positive at 0.35, 1, 3 and 10 µg/ml	Min. 98%	Fekadu et al., 1994
DNA damage (*E. coli* uvrB/recA lac$^+$ vs. uvr$^+$/rec$^+$ lac$^-$)	In vitro/in vivo: Differential DNA repair in host-mediated assay: mouse, 40 or 200 mg/kg bw by gavage	+		Positive at 200 mg/kg bw in indicator bacteria isolated from stomach, lung, intestine, liver, kidney, spleen; only marginal not statistically significant effects in all organs at 40 mg/kg bw	Min. 98%	Fekadu et al., 1994
Gene mutation; mammalian cells						
Forward mutation (L5178Y mouse lymphoma cells / TK locus)	Mouse lymphoma assay: 0.625-10 µg/ml (+ S-9 mix); 0.0313-4 µg/ml (- S-9 mix)	+	(+)	a) With S-9 mix: clearly positive at 2.5 and 10 µg/plate (cytotox. at 10 µg/ml) b) Without S-9 mix: weakly positive at 0.0313, 0.25 µg/ml; positive at cytotoxic concentrations of 0.5 and 1 µg/ml)	> 99%	BASF, 1983
Forward mutation (Chinese hamster ovary cells)	HPRT assay: 11.8-47.3 µM (= 2-8 µg/ml) (- S-9 mix)	0	+	Positive at 4, 6 and 8 µg/ml (cytotoxic at 8 µg/ml)	99% (Source: Sigma-Aldrich)	Jansson et al., 1995
DNA damage and repair; mammalian cells						
DNA-Strand Breaks; Alkali labile sites (Chinese hamster ovary cells)	Alkaline Single Cell Gel / Comet Assay: 3.6-118.4 µM (= 0.6-20 µg/ml Comparative study on several chlorohydroxyfuranones including MCA and MX	0	+	Positive at 5, 10 and 20 µg/ml (non-cytotoxic resp. low-cytotoxic concentrations; viability > 75%))	99% (Source: Sigma-Aldrich)	Mäki-Paakanen et al., 2001
DNA-Strand Brakes (Chinese hamster ovary cells)	Sister-Chromatid Exchange: 1.5-8.9 µM (=0.25-1.5 µg/ml) Comparative study on several chlorohydroxyfuranones including MCA and MX	0	+	Positive at 1.5 µg/ml	99% (Source: Sigma-Aldrich)	Mäki-Paakanen et al., 2001

Table 3 (cont.): Overview of *in vitro* genotoxicity studies on MCA (key studies)

End-point (species)	Protocol	with[a]	without	Results Remarks	Purity of MCA	Reference
DNA-Repair (Primary rat hepatocytes	Unscheduled DNA synthesis: 10.24-25 µM (=1.7-4.2 µg/ml) Comparative study on several chloro-hydroxyfuranones including MCA and MX	0	+	Positive at 2.2, 2.7 and 3.4 µg/ml; 4.2 µg/ml cytotoxic concentration	99% (Source: Sigma-Aldrich)	Le Curieux et al., 1999
Chromosome mutation; mammalian cells						
Cytogenetic assay (Chinese hamster ovary cells)	Chromosome Aberration Test: 1.5-14.8 µM (= 0.25-2.5 µg/ml) Comparative study on several chloro-hydroxyfuranones including MCA and MX	0	+	Positive at 2 and 2.5 µM tested up to cytotoxic concentration	99% (Source: Sigma-Aldrich)	Mäki-Paakanen et al., 2001
Cytogenetic assay (Mouse lymphoma cells)	Microscale micronucleus assay: 1.56-25 µM (=0.26-4.2 µg/ml) Comparative study on several chloro-hydroxyfuranones including MCA and MX	0	(+)	Significantly positive only at relatively cytotoxic concentration 4.2 µg/ml (survival 35.3% of control)	99% (Source: Sigma-Aldrich)	Le Curieux et al., 1999
Cytogenetic assay (V79 Chinese hamster lung cells)	Micronucleus assay (acc. to proposal for OECD guidelines): 0.625 - 10 µg/ml (+ S-9 mix); 0.313 - 6 µg/ml (- S-9 mix)	-	+	a) With S-9 mix: negative (mean micronucleus frequency 0.65 - 1.25% vs. 0.7% in control and 0.9±0.3% in historical controls) b) Without S-9 mix: positive at ≥4 µg/ml (mean micronucleus frequency: 2.45%, 4.2%, 3.9% at 4, 5 and 6 µg/ml, resp., vs. 0.55% in control) All experiments: no cyto-toxicity (≥25 µg/ml +/- S-9 mix in pretests); no sup-pression of mitotic index Tests for aneugenic effects negative.	99.3%	BASF, 2001b

[a] with/without metabolic activation; - negative; + positive; (+) weakly positive; 0 not tested;

[b] bovine serum albumin

Bacterial gene mutation assays

MCA is a directly acting mutagen in bacterial gene mutation assays (standard plate Ames tests). In tests with *Salmonella typhimurium* without S-9 mix, the substance was mutagenic with strains TA

98, TA 100, and TA 1535, but not with TA 1537 and TA 1538, whilst all tests with S-9 mix were negative (BASF, 1981i). In Ames tests with strain TA 100, MCA was positive with and without metabolic activation (BASF, 1985; Ishiguro et al., 1988). Hytinnen et al. (1995) and Knasmüller et al. (1996) investigated the mutational spectra induced by MCA and other chlorohydroxyfuranones in the Ames tester strain TA 100. MCA induced primarily GC→AT transitions with a 4:1 preference for the second position of the his G46 codon.

The product of the reaction of MCA with glutathione was found to be not genotoxic in the Ames test (LaLonde and Xie, 1993). From the reaction products of MCA with N-acetylcysteine and cysteine, some were more mutagenic and some were less mutagenic than MCA (LaLonde and Xie, 1992; LaLonde et al., 1993).

Bacterial DNA repair assay

In a bacterial differential DNA repair assay with *E. coli* K-12 strains that differ in their repair capacity (uvrB/recA lac^+ and and uvr^+/rec^+ lac^-) a pronounced induction of repairable DNA damage was noted without metabolic activation. Addition of metabolic activation systems (mouse S-9 mix or bovine serum albumin) resulted in an almost complete loss of the DNA damaging activity of MCA (Fekadu et al., 1994).

Mammalian cell gene mutation assays

In a mouse lymphoma assay, MCA was considered weakly mutagenic without metabolic activation, because small (less than factor of 2), but repeatable increases in mutant frequencies were noted at concentrations of 0.013 and 0.25 µg/ml; at higher concentrations positive results only occurred at highly cytotoxic concentrations. With metabolic activation MCA was clearly mutagenic (BASF, 1983). Because this study was not designed to determine also small colony mutants, no conclusions can be drawn as to the concomitant induction of chromosomal aberrations in the mouse lymphoma assay.

In an HPRT assay with CHO cells, a concentration-dependent increase of the mutant frequency was observed without S-9 mix. The influence of metabolic activation was not tested (Jansson et al., 1995).

Mammalian cell DNA damage and repair assays and SCE assays

In an alkaline single cell gel / Comet assay in CHO cells MCA showed positive effects - depending on the endpoint taking into account from 5 up to 20 µg/ml (Mäki-Paakkanen et al., 2001). These concentrations were of no or low cytotoxicity in this test system. In a sister chromatid exchange assay performed in the same cell system MCA was only weakly positive in one of two experiments performed at the highest concentration tested (1.5 µg/ml), while in the first experiment only a slight dose related but insignificantly increase was seen (Mäki-Paakkanen et al., 2001). The test was performed up to cytotoxic concentrations as determined by the decrease of metaphases or in the frequency of second-division cells on the slides. Repair of DNA damage was investigated by Le Curieux et al. (1999) in an UDS assay in primary rat hepatocytes. In this assay MCA was positive at concentrations from 2.2 up to 3.4 µg/ml. 4.2 µg/ml was determined as the cytotoxic concentration (5.9 % survival of the control).

Micronucleus assay in mammalian cells

In a micronucleus assay with V79 Chinese hamster lung cells, MCA showed clastogenic activity without metabolic activation at non-cytotoxic concentrations. Tests for aneugenic effects using the mitotic shake off method were negative indicating true clastogenic activity of MCA. No clastogenic effects were seen in trials with metabolic activation (BASF 2001b). In another so called microscale micronucleus assay in mouse lymphoma cells MCA was only significantly positive at the highest

concentration tested that showed already a distinct cytotoxicity of 35.3 % survival compared to the control (Le Curieux et al. 1999).

Other in vitro genotoxicity studies

Adduct formation with the DNA bases adenosine, cytidine and guanosine has been shown *in vitro* (Kronberg et al., 1992; Kronberg et al., 1993; Asplund et al., 1995; Kronberg et al., 1996; Le Curieux et al., 1997). The products were identified as 3-(2'-deoxyribofuranosyl)-7-formylimidazo[2,1-i]purine (Le Curieux et al., 1997), chloropropenal derivatives of adenosine and cytidine (Kronberg et al. 1996), etheno derivatives of adenosine, cytidine and guanosine (Kronberg et al., 1992), ethanocarbaldehyde derivatives of adenosine and cytidine (Kronberg et al., 1993) and adenosinylethenoadenosine derivatives of adenosine (Asplund et al., 1995). The later products were postulated to be formed by oxidative properties of MCA. The formation of the chloroprenal derivatives, ethanocarbaldehyde derivatives and etheno derivatives from MCA is explained by an initial formation of mucoxychloric acid, which may be further broken down to chloroacetaldehyde, which could proceed via the chloromalonaldehyde that reacts with the nucleosides and forms subsequently the derivatives (Kronberg et al., 1996).

LaLonde and Ramdayal (1997) demonstrated the induction of single strand breaks in ΦX174 supercoiled plasmid DNA, which was transformed into relaxed and linear DNA. Increasing concentrations of glutathione diminished the cleavage of the supercoiled DNA.

In vitro / in vivo Studies

Fekadu et al. (1994) tested the DNA damaging activity of MCA (purity 98 %) in a host-mediated assay using the same *E. coli* strains as those used in their in vitro DNA repair test (see above). After i.v. injection of these *E. coli* strains a single dose of 200 mg/kg bw of MCA (purity 98 %) was administered to Swiss mice by gavage; the mice were sacrificed after 2 hours. Statistically significant induction of repairable DNA damage was found in all examined organs, i.e. stomach, lung, liver, intestine, kidney and spleen. In a second experiment with a dose of 40 mg/kg bw, only marginal effects were noted. The genotoxic response in the host-mediated assay was considered weaker than in the corresponding *in vitro* bacterial test system without metabolic activation, possibly because MCA is inactivated by non-specific protein binding or metabolic detoxification as also indicated by the loss of genotoxic activity in the vitro assay when metabolic activation systems were used (see above). However, the assay indicates that MCA or genotoxic active metabolites do reach various organs and may as well induce genotoxic effects *in vivo*.

In vivo Studies

In vivo, MCA caused a slight, but statistically significant increase in the incidence of total nuclear anomalies in the duodenum of B6C3F1 mice after a single oral exposure (Daniel et al., 1991). The purity of the MCA obtained from Aldrich was at least 98 %. The nuclear anomalies included micronuclei, pyknotic nuclei and karyorrhectic nuclei, however, a detailed description on the results of the individual anomalies is not given. The increase was seen in the duodenum only at the intermediate (0.36 mmol/kg = 60.8 mg/kg bw) and highest dose (0.46 mmol/kg = 79.4 mg/kg bw). With regard to micronuclei MCA induced micronuclei in the duodenum of mice in one animal out of ten per dose group after single oral doses (38.9, 60.8, and 79.4 mg/kg bw). Since this is the only parameter of the study that can be directly attributed to genotoxicity, the *in vivo* genotoxic effect of MCA in this study is considered to be equivocal. No further reliable reports on the *in vivo* genotoxicity of MCA are available.

Studies in Humans

Chromosome analyses were performed in 30 workers handling MCA (Fleig and Zober, 1989). Exposure period was 11.9 years (median, range 1-17 years). Measurements of concentrations at the workplace were not available. Comparison of the structural aberrations showed no significant difference between exposed and control groups.

Conclusion

In vitro, MCA is a direct acting mutagen and clastogen in mammalian and bacterial cells, and forms exocyclic DNA adducts. *In vivo*, MCA caused a slight, but statistically significant increase in the incidence of total nuclear anomalies (including micronuclei, pyknotic nuclei and karyorrhectic nuclei) in the duodenum of mice after a single oral exposure to 60.8 and 79.4 mg/kg bw. MCA induced micronuclei in one animal out of ten per dose group in the duodenum of mice after single oral doses (38.9, 60.8, and 79.4 mg/kg bw). Based on the available *in vitro* and *in vivo* data, it can be concluded that MCA has a genotoxic potential.

3.1.7 Carcinogenicity

In vivo Studies

Oral

In an oral study with two different hybrid mouse strains, administration of about 7 mg MCA ("commercial source", not specified) per kg bw per day with the diet for 18 months did not cause an increase in tumor rates (Innes et al., 1969). Limitations of this study are: only one dose used though maximum tolerated dose; limited number of animals (18 per sex and group); limited number of organs examined; limited tumor categories, i.e. hepatomas, pulmonary tumors, lymphomas, and total mice with tumors.

Indirect evidence for the lack of a carcinogenic potential of MCA (purity >98 %) with respect to colon cancer has been provided by short-time bioassays, in which the induction of aberrant crypt foci (ACF) being considered as preneoplastic lesions was studied. MCA (>98 % purity) applied with the drinking water at dose levels of 0.45 and 0.90 mg/ml (corresponding to 43 and 77 mg/kg bw/day, respectively) over 6 weeks to Fisher F334 rats, neither induced ACF nor enhanced the number of ACF per colon or the ratio of aberrant crypts per ACF in animals pretreated with the potent colon carcinogen 1,2-dimethylhydrazine. In mice, small, but statistically non-significant inducing effects were noted using principally the same study design but MCA application via the drinking water at dose levels of 0.18 and 0.35 mg/ml (corresponding to 27 and 54 mg/kg bw/day, respectively) for 4 weeks with a subsequent recovery period of 12 weeks (Steffensen et al., 1999).

Structure activity predictions of the cancer potential of drinking water disinfection by-products, based on human expert judgment and input from the OncoLogic expert system gave a “moderate concern” level for MCA (Woo et al., 2002).

Conclusion

MCA did not induce aberrant crypt foci or intestinal tumors when given in drinking water at dose levels of 0.45 and 0.9 mg/ml over 6 weeks to rats or at dose levels of 0.18 and 0.35 mg/ml over 4 weeks with subsequent 12-weeks recovery to mice, respectively. The available data for MCA are not sufficient to judge the carcinogenic potential. Given the available data for genotoxicity there are, however, concerns with regard to this endpoint.

3.1.8 Toxicity for Reproduction

Effects on Fertility

There are no fertility studies available.

MCA is a corrosive substance used mainly in closed systems as a chemical intermediate. Transport of the isolated material is controlled and is limited to a very small number of sites. Exposure is controlled in occupational settings and is negligible for consumers.

Because of its corrosive properties, and the limited exposure potential, animal tests with MCA for its effects on fertility were not performed.

Developmental Toxicity

In a prenatal developmental toxicity study conducted in accordance with OECD guideline 414 (BASF 2001a), female Sprague-Dawley rats received dose levels of 5, 30 or 60 mg/kg bw/day by gavage on days 6 to 19 post coitum. The animals were sacrificed on day 20 p.c. The NOAEL for maternal toxicity was 5 mg/kg bw/day based on reduced food consumption and body weight gain at 30 and 60 mg/kg bw/day (see further details in section 3.1.5). Uterus weight was slightly increased in all treated groups due to higher litter sizes, which was considered to be by chance and, thus, of no biological significance. Conception rates were 92 % in control group, 80 % in 5 mg/kg bw/day group, 76 % in 30 mg/kg bw/day group and 72 % in 60 mg/kg bw/day group; no substance-related and/or biologically relevant differences between all test groups were noted regarding mean number of copora lutea and implantation sites or in the values calculated for pre and post-implantation losses, number of resorptions and viable fetuses. A slightly higher number of fetuses per litter in treated groups is considered to be by chance and, thus, of no biological significance. There were no substance-induced indications of teratogenicity up to and including the highest dose level (60 mg/kg bw/day), i.e. examination of fetuses did not reveal any substance-related effects on sex ratio, weights of fetuses, external malformations, external variations, soft tissue malformations, soft tissue variations, skeletal malformations, skeletal variations or fetal skeletal cartilage examination. Scattered occurrence of external, soft tissue and skeletal malformations and variations throughout all test groups, including controls, did not suggest any relation to treatment with the substance because of their low incidence, absence of dose-response-relationship and/or statistical significance.

Conclusion

Because of its corrosive properties, and the very limited exposure potential, animal tests with MCA for its effects on fertility were not performed. In an oral developmental study performed in accordance with OECD TG 414 in rats, the NOAEL for maternal toxicity was 5 mg/kg bw/day. The NOAEL for developmental toxicity was 60 mg/kg bw/day, which was the highest dose level applied. There were no signs of developmental toxicity or teratogenicity.

3.2 Initial Assessment for Human Health

There are no reliable experimental data on the toxicokinetic behavior of mucochloric acid (MCA) *in vivo* available. From the results of acute toxicity studies, it is very likely that MCA itself or its metabolites are systemically available after oral exposure. *In vitro*, MCA reacted with N-acetylcysteine, cysteine and glutathione (GSH).

The acute toxicity (LD_{50}) of MCA was between 300 and 400 mg/kg bw in rats after oral exposure and >200 mg/kg bw (highest tested dose) in rabbits after dermal exposure. The LC_{50} after 4-hour inhalation exposure of rats was >5.1 mg/l (highest tested concentration). Clinical signs included

atonia and ataxia after oral exposure, preening, dyspnoea and salivation during inhalation and skin irritation after dermal exposure.

MCA is corrosive to the rabbit skin and eye. A guinea pig sensitization test was negative, but limited experience from occupational exposure in humans indicates a skin sensitizing potential of MCA.

There is limited data on repeated dose toxicity available, indicating that irritant/corrosive effects at the site of first contact are the main effects to be expected after repeated exposure. In pregnant rats, no systemic target organ has been identified after oral exposure from day 6 to 19 p.c. (LOAEL: 30 mg/kg bw/day, based on reduced food consumption and body weight gain together with minor clinical symptoms (ptyalism) and whitish foci in the stomach interpreted as local effects due to the corrosive properties of MCA; NOAEL: 5 mg/kg bw/day). No target organ was identified in mice after dietary exposure to 7 mg/kg bw/day for 18 months (only one dose tested). Because of the limited exposure potential and the availability of reliable, though limited, data on repeat dose toxicity, no further animal testing is warranted.

In vitro, MCA is a direct acting mutagen and clastogen in mammalian and bacterial cells, and forms exocyclic DNA adducts. *In vivo*, mucochloric acid caused a slight, but statistically significant increase in the incidence of total nuclear anomalies (including micronuclei, pyknotic nuclei and karyorrhectic nuclei) in the duodenum of mice after a single oral exposure to 60.8 and 79.4 mg/kg bw. MCA induced micronuclei in one animal out of ten per dose group in the duodenum of mice after single oral doses (38.9, 60.8, and 79.4 mg/kg bw). Based on the available *in vitro* and *in vivo* data, it can be concluded that MCA has a genotoxic potential.

Because of its corrosive properties, and the very limited exposure potential, animal tests with MCA for its effects on fertility were not performed. In an oral developmental study performed in accordance with OECD TG 414 in rats, the NOAEL for maternal toxicity was 5 mg/kg bw/day. The NOAEL for developmental toxicity was 60 mg/kg bw/day, which was the highest dose level applied. There were no signs of developmental toxicity or teratogenicity.

MCA did not induce aberrant crypt foci or intestinal tumors when given in drinking water at dose levels of 0.45 and 0.9 mg/ml (corresponding to 43 and 77 mg/kg bw/day, respectively) over 6 weeks to rats or at dose levels of 0.18 and 0.35 mg/ml over 4 weeks with subsequent 12-weeks recovery to mice, respectively. The available data for MCA are not sufficient to judge its carcinogenicity. Given the available data for genotoxicity there are, however, concerns with regard to this endpoint.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

MCA was tested in a limited number of aquatic species.

One valid short-term toxicity study carried out according to current protocols is available for each trophic level as given in Table 4 below:

Table 4: Short -term toxicity of MCA in aquatic organisms (key-studies)

Organism	Species	Value	Reference
Fish	*Leuciscus idus*	LC_{50} (96 h) = 123 mg/l	BASF, 1988b
Invertebrates	*Daphnia magna*	EC_{50} (48 h) = 13 mg/l	BASF, 1988c
Algae	*Scenedesmus subspicatus*	E_bC_{50} (72 h) = 62 mg/l E_rC_{50} (72 h) = 65 mg/l	BASF, 1988d
Bacteria	*Pseudomonas putida*	EC_{50} (17 h) = 6.4 mg/l	BASF, 1988e
Activated sludge, industrial	*activated sludge*	EC_{20} (0,5 h) > 2000 mg/l EC_{50} (0.5 h) = 700 mg/l	BASF, 1981j

All tests were performed in static systems and the effect values are related to nominal concentrations. Although no analytical monitoring was performed, these nominal values can be considered reliable, because the test substance is not very volatile from water and no biodegradation is expected to occur within the test duration. In the tests with algae and bacteria, the toxic effects might have been influenced by the acidity of the test substance, which was not completely neutralized in these tests.

The predicted no effect concentration (PNEC) can be based on the lowest effect value (13 mg/l for *Daphnia magna*). A $PNEC_{aqua}$ of 13 µg/l can be derived by applying an assessment factor of 1000 according to the EU Technical Guidance Document (TGD, 1996).

4.2 Terrestrial Effects

No data are available on terrestrial organisms.

4.3 Other Environmental Effects

There are no data available.

4.4 Initial Assessment for the Environment

The distribution modelling using *Mackay*, Level I, indicates water to be the almost exclusive target compartment. The substance has no considerable potential for bioaccumulation (log K_{OW} = 0.697). It is not inherently biodegradable. In a number of Zahn-Wellens tests which were run considerably longer than 28 days, elimination of the test substance started after a lag phase of several days, regardless of whether adapted or non-adapted activated sludge was used. Hence, MCA can be regarded as partially biodegradable after an appropriate adaptation. Negative effects on the degradation activity of activated sludge are possible. From the structure of MCA hydrolysis is not to be expected.. Photodegradation in air is to be expected under environmental conditions with a half-life of 21.4 h.

Aquatic effects data are available for three trophic levels (fish: LC_{50} (96 h) = 123 mg/l; crustacea: EC_{50} (48 h) = 13 mg/l; algae: E_bC_{50} (72 h) = 62 mg/l; E_rC_{50} (72 h) = 65 mg/l). A $PNEC_{aqua}$ of 13 µg/l can be derived based on the lowest toxicity value (EC_{50} 13 mg/l) found for *Daphnia magna*.

No data are available on terrestrial organisms, but no significant exposure to MCA is expected either.

5 RECOMMENDATIONS

Human Health:

The chemical is currently of low priority for further work. The chemical possesses properties indicating a hazard for human health (corrosivity, genotoxicity, potential carcinogenicity), but controls for occupational exposure are in place in OECD countries. In view of concerns that the chemical may be a genotoxic carcinogen, there is a recommendation for sharing the toxicological and exposure data with regulatory agencies responsible for drinking water, because traces of MCA can occur in drinking water as a disinfection by-product. Based on this, countries may want to consider toxicokinetic/metabolism studies, and, if then indicated, further studies relating to the carcinogenicity endpoint.

Environment:

The chemical is currently of low priority for further work. The chemical possesses properties indicating a hazard for the environment. Based on data presented by the Sponsor country, exposure to the environment is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

6 REFERENCES

Asplund D, Konberg L, Sjöholm R, Munter T (1995). Reaction of mucochloric acid with adenosine: formation of 8-(N6-adenosinyl)ethenoadenosine derivatives. Chem. Res. Toxicol. **8**, 841-846.

BASF (1960a) Acute toxicity and skin/eye irritation (X/211). BASF AG, Department of Toxicology, 09.09.1960 (unpublished data).

BASF (1960b). Acute toxicity and skin/eye irritation (X/221). BASF AG, Department of Toxicology, 09.09.1960 (unpublished data).

BASF (1961). Acute toxicity and skin/eye irritation (X/211; X/329). BASF AG, Department of Toxicology, 13.02.1961 (unpublished data).

BASF (1964a). Acute toxicity in rats (XIV/182-184). BASF AG, Department of Toxicology, 22.09.1964 (unpublished data).

BASF (1964b). Skin irritation in rabbits (XIV/97; XIV/182-184). BASF AG, Department of Toxicology, 13.08.1964 (unpublished data).

BASF (1964c). Skin sensitization in guinea pigs (XIV/97; XIV/182-183). BASF AG, Department of Toxicology, 15.09.1964 (unpublished data).

BASF (1970). Safety data sheet Mucochlorsaeure, D 034, BASF AG, July 1970 (unpublished data).

BASF (1976). Sicherheitstechnische Kenndaten, TLM/SIK 75/1379, BASF AG, 21.05.1976 (unpublished data).

BASF (1977). Acute dermal toxicity in rabbits (77/164 acute dermal toxicity). BASF AG, Department of Toxicology, 16.08.1977 (unpublished data).

BASF (1980a). Acute 4-hour inhalation toxicity in rats (77/164). BASF AG, Department of Toxicology, 24.12.1980 (unpublished data).

BASF (1980b). Corrosivity in rabbits (77/164). BASF AG, Department of Toxicology, 06.05.1980 (unpublished data).

BASF (1981a). Respirometric test (mucochloric acid purified technical grade). BASF AG, Department of Ecology, 15.09.1981 (unpublished data; Reprint 18.07.2002).

BASF (1981b). Respirometric test (mucochloric acid purified technical grade; adapted inoculum). BASF AG, Department of Ecology, 21.08.1981 (unpublished data; Reprint of 18.07.2002).

BASF (1981c). Zahn-Wellens test (mucochloric acid; adapted inoculum). BASF AG, Department of Ecology (unpublished raw data of 11.06.1981 + unpublished reprint of 05.07.2002).

BASF (1981d). Zahn-Wellens test (mucochloric acid; adapted inoculum). BASF AG, Department of Ecology (unpublished raw data of 14.08.1981 + unpublished reprint of 05.07.2002).

BASF (1981e). Zahn-Wellens test (mucochloric acid). BASF AG, Department of Ecology, 10.12.1981 (unpublished data; Reprint 05.07.2002).

BASF (1981f). Zahn-Wellens test (mucochloric acid). BASF AG, Department of Ecology, 14.04.1981 (unpublished data; Reprint 05.07.2002).

BASF (1981g). Zahn-Wellens test (mucochloric acid). BASF AG, Department of Ecology, 07.05.1981 (unpublished data; Reprint 05.07.2002).

BASF (1981h). Biochemical oxygen demand in 5 days (BOD5) (mucochloric acid). BASF AG, Department of Ecology, 04.05.1981 (unpublished data; Reprint 16.07.2002).

BASF (1981i). Report on the study of Mucochlorsaeure techn. trocken (80/408) in the Ames test. BASF AG, Department of Toxicology, 14.07.1981 (unpublished data).

BASF (1981j) Short-term respiration inhibition test with BASF activated sludge. BASF AG, Department of Ecology, 07.04.1981(unpublished data)

BASF (1982). Zahn-Wellens test (mucochloric acid). BASF AG, Department of Ecology, 03.02.1982 (unpublished data; Reprint 05.07.2002).

BASF (1983). Mutagenicity evaluation of Mucochlorsaeure in the mouse lymphoma forward mutation assay (82/259). BASF AG, Department of Toxicology, 02.08.1983 (unpublished data).

BASF (1985). Report on the study of Mucochlorsaeure techn. rein (84/212) in the Ames test. BASF AG, Department of Toxicology, 13.05.1985 (unpublished data).

BASF (1988a). Partition coefficient, report to J.Nr.124837/01. BASF AG, Analytical Laboratory, 03.03.1988 (unpublished data).

BASF (1988b). Acute toxicity to *Leuciscus idus* (88/16). BASF AG, Department of Toxicology, 09.09.1988 (unpublished data).

BASF (1988c). Acute toxicity to Daphnia magna (0040/88). BASF AG, Department of Ecology, 11.02.1988 (unpublished data).

BASF (1988d). Toxicity to *Scenedesmus subspicatus* (0040/88). BASF AG, Department of Ecology, 01.03.1988 (unpublished data).

BASF (1988e). Toxicity to *Pseudomonas putida* (0040/88). BASF AG, Department of Ecology, 10.06.1988 (unpublished data).

BASF (1998). Department of Ecology, 20.12.1998 (unpublished data).

BASF (1999a). Safety data sheet Mucochlorsaeure techn. rein. BASF AG, 06.01.1999 (unpublished data).

BASF (1999b). Sicherheitstechnik, interne Mitteilung (internal note), BASF AG, 05.11.1999 (unpublished data).

BASF (1999c). Calculation of photodegradation in air. BASF AG, Department of Ecology, 16.11.1999 (unpublished data).

BASF (2000). Safety data sheet MUCOCHLORSAEURE TECHN., 19.09.2000 (unpublished data).

BASF (2001a). Prenatal developmental toxicity study in Sprague-Dawley rats after oral administration, Report No.: 30R0345/999062. BASF AG, Product Safety, Regulations, Toxicology and Ecology 12.11.2001 (unpublished data).

BASF (2001b). In vitro micronucleus assay with mucochloric acid techn. pure in V79 cells, Report No.: 33M0345/994185. BASF AG, Product Safety, Regulations, Toxicology and Ecology (unpublished data).

BASF (2002a). Calculation of vapour pressure with MPBPWIN v 1.40. BASF AG, Product Safety, Regulations, Toxicology and Ecology 19.07.2002 (unpublished data).

BASF (2002b). Calculation of Mackay Level I v0.9 with BASF programme. Maisch R, BASF AG (1999). (programme developer) 22.07.2002 (unpublished data).

BASF (2002c). Calculation of Mackay Level I with Trent University programme v2.11. BASF AG, Product Safety, Regulations, Toxicology and Ecology 23.07.2002 (unpublished data).

BASF (2003). Calculation of photodegradation in air. BASF AG, Department of Ecology, 05.08.2003 (unpublished data).

Daniel FB, Olson GR and Stober JA (1991). Induction of gastrointestinal tract nuclear anomalies in B6C3F1 mice by 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and 3,4-(dichloro)-5-hydroxy-2(5H)-furanone, mutagenic byproducts of chlorine disinfection. Environ. Mol. Mutagen. **17,** 32-39.

Danish Product Register (2002). February 2002.

Fekadu K, Parzefall W, Kronberg L, Franzen R, Schulte-Hermann R and Knasmueller S (1994). Induction of genotoxic effects by chlorohydroxyfurane, byproducts of water disinfection, in E. coli K.12 cells recovered from various organs in mice. Environ. Molec. Mutagen. **24**, 317-324.

Fleig I and Zober A (1989). The use of cytogenetic monitoring in occupational medicine: a summary of chromosome analyses. Poster presented at: Biomonitoring and carcinogen risk assessment, Queen's College, Cambridge, England, 27-28 July 1989.

Hemming J, Holbom B, Reunanen M and Tikkanen L (1986). Determination of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in chlorinated drinking and humic waters. Chemosphere **15**, 549-556.

Hommel G (1992). Handbuch der gefaehrlichen Gueter. Merkblatt 1284. Springer-Verlag, 1992.

Hytinnen JMT, Niittykoski M and Jansson K (1995). Lack of uniformity in the mutational spectra of chlorohydroxyfuranones in *S. typhimurium* strain TA 100. Mutagen. **10**, 321-323.

Innes JRM, Ulland BM, Valerio MG, Petrucelli L, Fishbein L, Hart ER, Palotta AJ, Bates RR, Falk HL, Gart JJ, Klein M, Mitchell I and Peters J (1969). Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J. Nat. Canc. Inst. **42**, 1101-1114.

Ishiguro Y, Santodonato J and Neal MW (1988). Mutagenic potency of chlorofuranones and related compounds in Salmonella. Environ Mol Mutagen **11**, 225-234.

Jansson K and Hyttinen JMT (1994). Induction of gene mutation in mammalian cells by 3-chlor-4-(cichlormethyl-5-hydroxy-2(5H)-furanone (MX) a chlorine disinfection by-product in drinking water. Mutat. Res. **322**, 129-132.

Jansson K, Hyttinen JMT, Niittykoski M and Mäki-Paakkanen J (1995). Mutagenicity in vitro of 3,4-dichloro-5-hydroxy-2(5H)-furanone (mucochloric acid), a chlorine disinfection by-product in drinking water. Environ. Mol. Mutagen. **25**, 284-287.

Knasmüller S, Zöhrer E, Kronberg L, Kundi M, Franzén R and Schulte-Hermann R (1996). Mutational spectra of Salmonella typhimurium revertants induced by chlorohydroxyfuranones, byproducts of chlorine disinfection of drinking water. Chem. Res. Toxicol. **9**, 374-381.

Kronberg L, Asplund D, Mäki J, Sjöholm R (1996). Reaction of mucochloric and mucobromic acids with adenosine and cytidine: formation of chloro- and bromopropenal derivatives. Chem. Res. Toxicol. **9**, 1257-1263.

Kronberg L and Franzén R (1993). Determination of chlorinated furanones, hydroxyfuranones, and butenedioic acids in chlorine treated water and in pulp bleaching liquor. Environ. Sci. Technol. **27**, 1811-1818.

Kronberg L, Karlsson S, Sjöholm R (1993). Formation of ethenocarbaldehyde derivatives of adenosine and cytidine in reactions with mucochloric acid. Chem. Res. Toxicol. **6**, 495-499.

Kronberg L, Sjöholm R and Karlsson S (1992). Formation of 3,N^4-ethenocytidine, 1,N^6-ethenoadenosine, and 1,N^2-ethenoguanosine in reactions of mucochloric acid with nucleosides. Chem. Res. Toxicol. **5,** 852-855.

LaLonde RT and Ramdayal F (1997). Mucochloric acid action on ΦX174 DNA: A comparison to other chlorine substituted 2(5H)-furanones. Chem. Res. Toxicol. **10**, 205-210.

LaLonde RT and Xie S (1992). A study of inactivation reactions of N-acetylcysteine with mucochloric acid, a mutagenic product of the chlorination of humic substances in water. Chem. Res. Toxicol. **5**, 618-624.

LaLonde RT and Xie S (1993). Glutathione and N-acetylcysteine inactivation of mutagenic 2(5H)-furanones form the chlorination of humics in water. Chem. Res. Toxicol. **6**, 445-451.

LaLonde RT, Xie S and Bu L (1993). Test of chiral recognition in the *salmonella typhimurium* (TA100) mutagenicity of mucochloric acid-cysteine adducts. Environ. Mol. Mutagen. **22**, 181-187.

LaLonde RT, Xie S, Chamulitrat W and Mason RP (1994). Oxidation and radical intermediates associated with the glutathion conjugation of mucochloric acid. Chem. Res. Toxicol. **7**, 482-486.

Le Curieux, Munter T and Kronberg L (1997). Identification of adenine adducts formed in reaction of calf thymus DNA with mutagenic chlorohydroxyfuranones found in drinking water. Chem. Res. Toxicol. **10**, 1180-1185.

Le Curieux F, Nesslany F, Munter T, Kronberg L and Marzin D (1999). Genotoxic activity of chlorohydroxyfuranones in the microscale micronucleus test on mouse lymphoma cells and the unscheduled DNA synthesis assay in rat hepatocytes. Mutagen. **14**, 457-462.

Mäki-Paakkanen J, Laaksonen M, Munter T, Kronberg L and Komulainen H (2001). Comparable DNA and Chromosome Damage in Chinese Hamster Ovary Cells by Chlorohydroxyfuranones. Environ. Mol. Mutagen. **38**, 297-305.

Mashkina OH and Bathisina GS (1971). Toxicology and hygienic standards for mucochloric acid in the air of a working zone. Gig. Tr. Prof. Zabol. **6**, 139-143. (in Russian with English abstract).

NCI (1968). Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrial chemicals. National Cancer Institute (PB-223159).

Patty FA (1967). Industrial Hygiene and Toxicology. 2nd rev. ed., Vol. II, Interscience Publ, 1975-1979.

Serjeant EP and Dempsey B (1979). Ionization constants of organic acids in aqueous solution. IUPAC Chemical Data Series No. 23, Pergamon Press, 1st ed., 81.

Smeds A, Vartiainen T, Mäki-Paakkanen J and Kronberg L (1999). Concentration of Ames mutagenic chlorohydroxyfuranones and related compounds in drinking waters. Environ. Sci. Technol. **31**, 1033-1039.

Steffensen IL, Paulsen JE, Engeset D, Kronberg L and Alexander J (1999). The drinking water chlorination by-products 3,4-dichloro-5-hydroxy-2[5H]-furanone (mucochloric acid) and 3-chloro-

4-(dichloromethyl)-5-hydroxy-2[5H]-furanone do not induce preneoplastic or neoplastic intestinal lesions in F344 rats, Balb/cA mice or C57BL/6J-Min mice. Pharmacol. Toxicol. **85**, 56-64.

Suzuki N and Nakaniski J. (1990). The determination of strong mutagen 3-chlor-4-(dichloro-methyl)-5-hydroxy-2(5)-furanone in drinking water in Japan. Chemosphere **21**, 387-392.

Swedish Product Register (2002). February 2002.

Swiss Product Register (2001). December 2001.

TGD (1996). Technical Guidance Documents in Support of the Commission Directive 93/67/EEC on Risk Assessment for New Substances and the Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances. European Chemicals Bureau, Ispra (1996)

WHO (2000). Environmental Health Criteria (EHC) Monograph No. 216 "Disinfectants and Disinfectant By-Products", International. Programme on Chemical Safety (IPCS), World Health Organisation.

WHO (2003). Guideline for drinking water quality 3rd edition - Draft chapter 8, World Health Organisation.

Woo YT, Lai D, McLain JL, Manibusan MK and Dellarco V (2002). Use of mechanism-based structure-activity-relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products. Environ. Health. Persp. **110**, 75-87.

Wright JM; Schwartz J, Varianinen T, Maki-Paakkanen J, Altshul L, Harrington JJ and Dockery DW (2002). 3-Chloro-4-(dichlormethyl)-5-hydroxy-2(5H)-furanone (MX) and mutagenic activity in Massachusetts drinking water. Environ. Health. Persp. **110**, 157-164.

I U C L I D

D a t a S e t

Existing Chemical ID: 87-56-9
CAS No. 87-56-9
EINECS Name mucochloric acid
EC No. 201-752-4
Molecular Weight 168.96 g/mol
Molecular Formula C4 H2 Cl2 O3

Producer Related Part
Company: BASF AG
Creation date: 29-NOV-2001

Substance Related Part
Company: BASF AG
Creation date: 29-NOV-2001

Memo: master

Printing date: 10-AUG-2004
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Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, SIDS

1.0.1 Applicant and Company Information

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Flag: Critical study for SIDS endpoint
19-NOV-2002

Type: cooperating company
Name: Oxon Italia S.p.A.
Country: Italy

Flag: Critical study for SIDS endpoint
19-NOV-2002

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: C4 H2 Cl2 O3
Mol. Weight: 168.96 g/mol

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002

1.1.1 General Substance Information

Substance type: organic
Physical status: solid
Purity: > 93 - 98 % w/w
Colour: colourless-yellowish
Odour: characteristic-pungent

Flag: non confidential, Critical study for SIDS endpoint
12-JUL-2004 (1)

Purity type: other: Mucochloric acid available for laboratory use: Mucochlorsaeure (Source Fluka)
Substance type: organic
Physical status: solid

Purity: >= 98 - % w/w

Reliability: (2) valid with restrictions
Technical Information provided by Sigma-Aldrich concerning compound sold from 1988 up to now; limitation no individual batch certificates available
Flag: Critical study for SIDS endpoint
07-JUL-2003 (2)

Purity type: other: Mucochloric acid available for laboratory use: Mucochlorsaeure 99% (T) (Source Sigma-Aldrich)
Substance type: organic
Physical status: solid
Purity: = 99 - % v/v

Reliability: (2) valid with restrictions
Technical Information provided by Sigma-Aldrich concerning compound sold from 1988 up to now; limitation no individual batch certificates available
Flag: Critical study for SIDS endpoint
07-JUL-2003 (2)

1.1.2 Spectra

1.2 Synonyms and Tradenames

.alpha.,.beta.-Dichloro-.beta.-formylacrylic acid

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

2,3-Dichloromaleic aldehyde acid

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

2-Butenoic acid, 2,3-dichloro-4-oxo-, (Z)- (9CI)

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

Dichloromalealdehydic acid

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

Malealdehydic acid, dichloro- (7CI, 8CI)

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

Mucochloric acid (6CI)

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

Mucochlorsaeure

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

1.3 Impurities

1.4 Additives

1.5 Total Quantity

Remark: production volume for the year 2000:

Germany : 1.000 - 5.000 t/a
EU : 1.000 - 5.000 t/a
Flag: non confidential, Critical study for SIDS endpoint
15-JUL-2003

1.6.1 Labelling

Labelling: provisionally by manufacturer/importer
Symbols: (C) corrosive
R-Phrases: (34) Causes burns
(22) Harmful if swallowed
(43) May cause sensitization by skin contact
(52/53) Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S-Phrases: (26) In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
(28) After contact with skin, wash immediately with plenty of soap and water
(36/37/39) Wear suitable protective clothing, gloves and eye/face protection
(45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
(61) Avoid release to the environment. Refer to special instructions/Safety data sets

Flag: non confidential, Critical study for SIDS endpoint
06-FEB-2004 (1)

1.6.2 Classification

Classified: provisionally by manufacturer/importer
Class of danger: corrosive
R-Phrases: (34) Causes burns

Flag: non confidential, Critical study for SIDS endpoint
06-FEB-2004 (1)

Classified: provisionally by manufacturer/importer
Class of danger: dangerous for the environment
R-Phrases: (52/53) Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment

Flag: non confidential, Critical study for SIDS endpoint
06-FEB-2004 (1)

Classified: provisionally by manufacturer/importer
Class of danger: harmful
R-Phrases: (21/22) Harmful in contact with skin and if swallowed

Flag: non confidential, Critical study for SIDS endpoint
06-FEB-2004 (1)

Classified: provisionally by manufacturer/importer
Class of danger: irritating
R-Phrases: (43) May cause sensitization by skin contact

Flag: non confidential, Critical study for SIDS endpoint
06-FEB-2004 (1)

1.6.3 Packaging

1.7 Use Pattern

Type: type
Category: Use in closed system

Flag: non confidential, Critical study for SIDS endpoint
21-SEP-1993

Type: industrial
Category: Chemical industry: used in synthesis

Flag: non confidential, Critical study for SIDS endpoint
21-SEP-1993

Type: use
Category: Intermediates

Flag: non confidential, Critical study for SIDS endpoint
21-SEP-1993

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

Type: Production

Remark: Mucochloric Acid is produced by reacting Furfural with Chlorine in the presence of water.
Flag: non confidential, Critical study for SIDS endpoint
28-MAY-2003

Type: Use

Remark: Mucochloric Acid is the starting material for the production of the active ingredients Chloridazon and Norfluorazon and for a series of plant protection products.
Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002

Orig. of Subst.: Synthesis
Type: Production

Remark: MCA is consumed in the process of synthesis of Chloridazon and Norflurazon except for trace amounts.
Flag: Critical study for SIDS endpoint
23-JUL-2004

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

Type of limit: MAK (DE)
Limit value: other: no MAK value established

Flag: non confidential, Critical study for SIDS endpoint
28-MAY-2003 (3)

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

Classified by: other: VwVwS (Germany) of 17.05.1999, Annex 2
Labelled by: other: VwVwS (Germany) of 17.05.1999, Annex 2
Class of danger: 2 (water polluting)

Remark: ID-Number: 1140
Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (4)

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. 201-752-4

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (5)

Type: ENCS
Additional Info: ENCS No. 2-1166

Remark: ENCS CLASSIFICATION:
Low Molecular Chain-like Organic Compounds.
Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (5)

Type: ECL
Additional Info: ECL Serial No. KE-10165

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (5)

Type: other: SWISS
Additional Info: SWISS No. G-3216

Remark: SWISS CLASSIFICATION:
Giftliste 1 (List of Toxic Substances 1), 31 May 1999.
Toxic Category 2: Acute oral lethal dose of 5 - 50 mg/kg.
Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (5)

Type: TSCA

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (5)

Type: PICCS

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (5)

Type: NDSL

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (5)

Type: AICS

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (5)

1.9.1 Degradation/Transformation Products

Type: thermal breakdown products
CAS-No: 630-08-0
EC-No: 211-128-3
EINECS-Name: carbon monoxide

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

Type: thermal breakdown products
CAS-No: 7647-01-0
EC-No: 231-595-7
EINECS-Name: hydrogen chloride

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

1.9.2 Components

1.10 Source of Exposure

1.11 Additional Remarks

1.12 Last Literature Search

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

06-FEB-2003

Chapters covered: 1
Date of Search: 18-FEB-2003

Remark: update 2003
18-FEB-2003

Chapters covered: 8
Date of Search: 18-FEB-2003

Remark: update 2003
18-FEB-2003

Type of Search: Internal and External
Chapters covered: 5
Date of Search: 18-MAY-2003

Remark: update 2003
23-JUN-2003

1.13 Reviews

2.1 Melting Point

Value: = 124 - 127 degree C

Test substance: other TS: no data, but presumably pure mucochloric acid

Reliability: (2) valid with restrictions
generally accepted handbook
Flag: Critical study for SIDS endpoint
09-JUL-2004 (6)

Value: = 125 - 127 degree C

Test substance: no data

Remark: range of melting
Reliability: (4) not assignable
Manufacturer / producer data without proof
31-JUL-2002 (7)

Value: = 125 - 127 degree C

Test substance: no data

Reliability: (4) not assignable
Manufacturer / producer data without proof
31-JUL-2002 (8)

Value: = 95 - 115 degree C
Decomposition: yes at degree C

Test substance: other TS: mucochloric acid, purified technical grade

Remark: Stable up to approximately 170 °C
Reliability: (4) not assignable
Manufacturer / producer data without proof
31-JUL-2002 (9)

2.2 Boiling Point

Value:

Method: other

Result: The substance is not stable beyond 170 °C (decomposition).
Flag: Critical study for SIDS endpoint
09-JUL-2004

2.3 Density

Type: bulk density
Value: = 750 - 800 kg/m3

Method: other: DIN 53 468
Test substance: no data

Reliability: (4) not assignable

Manufacturer / producer data without proof
Flag: Critical study for SIDS endpoint
27-JUL-2002 (7)

Type: bulk density
Value: = 950 kg/m3

Test substance: other TS: mucochloric acid, purified technical grade

Reliability: (4) not assignable
Manufacturer / producer data without proof
Flag: Critical study for SIDS endpoint
27-JUL-2002 (9)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = .00139 hPa at 25 degree C

Method: other (calculated): MPBPWIN v.1.40, US EPA (2000), modified Grain method
Test substance: other TS: Mucochloric acid, pure (calculation!)

Remark: Input data:
- Melting point: 127 °C (user entered)
- Boiling point (estimated): 267.31 °C

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
30-JUL-2002 (10)

2.5 Partition Coefficient

log Pow: = .697 at 25 degree C

Method: other (measured)
GLP: no

Method: In quadruplicate determinations, test vessels were prepared containing accurately measured amounts of mucochloric acid together with 25.0 ml octanol-1 and 25 ml aqua dest. After achieving equilibrium the aqueous phase was separated (no further information) and diluted with dimethylformamide (DMF). In this sample, the concentration of MCA was determined by gas chromatography (concentration of external standard: 0.4514& (m(m) MCA in water/DMF). The concentrations of MCA in the octanol phase was calculated based on mass balance.
Result: Results of the four determinations:
Vessel #1: Pow 4.34; log Pow 0.637
Vessel #2: Pow 4.42; log Pow 0.645
Vessel #3: Pow 5.56; log Pow 0.745
Vessel #4: Pow 5.75; log Pow 0.760

Mean/Standard deviation:
Pow 5.02 +/- 0.74; log Pow 0.697
Reliability: (2) valid with restrictions

Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; concentration of test substance determined only in one phase.

Flag: Critical study for SIDS endpoint

29-JUL-2002 (11)

log Pow: = .259

Method: other (calculated): Increment method by Rekker with computer programme of firm CompuDrug Ltd.

Reliability: (2) valid with restrictions
Calculated value in accordance with generally accepted standard methods

29-JUL-2002 (12)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 27 g/l at 20 degree C

Reliability: (2) valid with restrictions
generally accepted handbook
Flag: Critical study for SIDS endpoint

09-JUL-2004 (6)

Solubility in: Water
Value: ca. 24 g/l at 20 degree C
pH value: 2.2
Conc.: 24 g/l at 20 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof

29-JUL-2002 (9)

2.6.2 Surface Tension

2.7 Flash Point

Value: = 100 degree C

Reliability: (2) valid with restrictions
generally accepted handbook
Flag: Critical study for SIDS endpoint

09-JUL-2004 (6)

Value: > 100 degree C

Method: other: DIN 51 758

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

06-NOV-2001 (7)

Value: > 127 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof
Flag: Critical study for SIDS endpoint
06-NOV-2001 (9)

2.8 Auto Flammability

Value:

Method: other: VDI 2263 part 1, 1.4.1

Result: not self heating
Reliability: (2) valid with restrictions
Restrictions: Discrepancy between documented test parameters and standard methods, but scientifically acceptable
Flag: Critical study for SIDS endpoint
29-JUL-2002 (8)

2.9 Flammability

Method: other: VDI 2263 part 1, 1.2

Result: not highly flammable
Reliability: (2) valid with restrictions
Restrictions: Discrepancy between documented test parameters and standard methods, but scientifically acceptable
Flag: Critical study for SIDS endpoint
29-JUL-2002 (8)

2.10 Explosive Properties

Method: other: comparable to 92/69/EEC, A 14

Result: not explosive
Reliability: (2) valid with restrictions
Restrictions: Discrepancy between documented test parameters and standard methods, but scientifically acceptable
Flag: Critical study for SIDS endpoint
29-JUL-2002 (8)

2.11 Oxidizing Properties

Result: no oxidizing properties

Remark: because of chemical structure
Reliability: (2) valid with restrictions
Expert judgement
Flag: Critical study for SIDS endpoint
06-NOV-2001 (13)

2.12 Dissociation Constant

Acid-base Const.: pKa = 4.20 at 25 °C

GLP: no data

Reliability: (2) valid with restrictions
generally accepted handbook
Flag: Critical study for SIDS endpoint
09-JUL-2004 (14)

2.13 Viscosity

2.14 Additional Remarks

Remark: Hazardous reactions:
exothermic reaction with alkalies
Reliability: (4) not assignable
Manufacturer / producer data without proof
Flag: Critical study for SIDS endpoint
06-NOV-2001 (9)

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Rate constant: = .0000000000179753 cm³/(molecule * sec)
Degradation: = 50 % after 21.4 hour(s)

Method: other (calculated): AOP (v1.90)
GLP: no
Test substance: other TS: Mucochloric acid, pure (calculation!)

Remark: Calculation based on a 24 h day.
Reliability: (2) valid with restrictions
accepted calculation method
Flag: Critical study for SIDS endpoint
09-JUL-2004 (15)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: O3
Conc. of sens.: 700000000000 molecule/cm³
Rate constant: = .00000000000000000057 cm³/(molecule * sec)
Degradation: = 50 % after 2001.5 day(s)

Method: other (calculated):AOP (v1.90)
GLP: no
Test substance: other TS: Mucochloric acid, pure (calculation!)

Reliability: (2) valid with restrictions
accepted calculation method
Flag: Critical study for SIDS endpoint
09-JUL-2004 (15)

3.1.2 Stability in Water

Type: abiotic

Result: Rate constants for hydrolysis (25 °C) cannot be estimated for this structure.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
27-JUL-2002 (16)

Type: abiotic

Result: Mucochloric acid exists in two isomeric forms depending upon the pH value of the aqueous medium so this equilibrium cannot be described as a hydrolysis. The two chlorine atoms attached to the C-C double bond are resistant to hydrolysis or other nucleophilic substitution reactions like all halogen atoms bound to C-C double bonds or benzene structures because of their extremely low reactivity.
The pH-dependant equilibrium as such does not represent a

hydrolysis and the hydrolytic cleavage of the chlorine atoms attached to the olefinic double bond is not possible because of lacking reactivity of these chlorine atoms.

Flag: Critical study for SIDS endpoint

09-JUL-2004

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

Medium: drinking water

Remark: MCA can be present in the aqueous environment as a result of chlorine bleaching and chlorine-disinfection of drinking water. Among other chlorinated organic compounds, MCA can be formed by reaction of chlorine with natural organic matter (NOM), particularly humic acids. In a determination of chlorinated furanones and hydroxyfuranones in pulp bleaching liquor, in chlorine-treated natural humic water and in Finnish chlorine-treated drinking water, Kronberg and Franzén (1993) detected MCA in nearly all extracts. In a sample of chlorination-stage bleaching liquor derived from pine craft pulp, which was prebleached with oxygen they detected 67 µg MCA/l. In natural humic water, MCA concentrations of about 2.5 µg/l were found after chlorination. Maximal concentrations of MCA found in chlorinated drinking waters were around 10 - 60 ng/l. In a more recent study Smeds et al. (1999) found levels up to 12 ng/l in several of 35 investigated Finnish and one Russian drinking water samples. For comparison, the related compound
3?chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone), generally known as MX, was found in the same range, i.e. at levels of 0.4-80 ng/l (Hemming et al. 1986; Suzuki and Nakaniski 1990; Kronberg and Franzén 1993; Wright et al. 2002). A detailed elaboration of this inadvertent formation of MCA is beyond the scope of this SIAR. A comprehensive review on disinfectants and disinfectant by-products was published by the WHO (2000). With regard to halogenated hydroxyfuranones, no data on levels of MCA are given.

29-APR-2004 (17) (18) (19) (20) (21)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Type: fugacity model level I
Media: other: air - biota - sediment(s) - soil - water
Method: other: Calculation according Mackay, Level I
Air: .03 **% (Fugacity Model Level I)**
Water: 99.89 **% (Fugacity Model Level I)**
Soil: .04 **% (Fugacity Model Level I)**

Method: Calculation programme by Maisch R (1992) version 0.9, BASF AG, based on the principles described in: Mackay D (1991) Multimedia environmental models: The fugacity approach.

Lewis Publ. Inc.

Input parameters:
- Temperature: 20 °C
- Molecular weight: 168.96 g/mol
- Vapour pressure: 0.00139 hPa
- Solubility in water: 27 g/l (159.8011 mol/m³)
- log Pow: 0.697
- Melting point: 125 °C
- Amount of chemical: 100 mol

Remark:

	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: Further results data:
- Sediment: 0.04 %
- Biota: 0.00024 %

Reliability: (2) valid with restrictions
accepted calculation method

Flag: Critical study for SIDS endpoint

09-JUL-2004 (22)

3.3.2 Distribution

Media: water - air
Method: other (calculation): HENRYWIN v3.10, US EPA (2000)

Method: Bond Estimation Method: calculation of Henry's Law Constant at 25 °C based on QSAR methods
Result: Henry's Law Constant: 8.77*10e-6 Pa*m3/mole (25 °C)
Reliability: (2) valid with restrictions
Accepted calculation method
Restrictions: acidity of MCA and transformation to ring form not taken into account.

09-JUL-2004 (23)

Media: water - air
Method: other (calculation): based on mol mass, vapour pressure and water solubility

Remark: Calculated by Mackay Level I programme with following input parameters:
- Mol mass: 168.96 g/mol
- Vapour pressure: 0.00139 hPa
- Water solubility: 27 g/l
- Temperature: 20 °C

Result: Henry's Law Constant: 8.6983*10E-4 Pa*m3/mole (25 °C)
Reliability: (2) valid with restrictions
Accepted calculation method
Restrictions: vapour pressure calculated
Flag: Critical study for SIDS endpoint

09-JUL-2004 (24)

Media: water - soil

Method: other (calculation): PCKOWIN, v1.6

Remark: The Koc of this structure may be sensitive to pH.
The estimated Koc reppresents a best-fit to the majority of experimental values; however, the Koc may vary significantly with pH.
Result: log Koc = 0.00, estimated Koc = 1
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
09-JUL-2004 (25)

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, industrial, adapted
Concentration: 100 mg/l related to DOC (Dissolved Organic Carbon)
358 mg/l related to Test substance
Contact time: 40 day(s)
Degradation: = 40 - 50 % after 40 day(s)
Result: other: under test conditions elimination (primarily biodegradation) after lag phase, but not inherently biodegradable according to criteria of OECD Guideline 302 B
Kinetic:
18 day(s) = 11 %
27 day(s) = 22 %
29 day(s) = 51 %
32 day(s) = 14 %
34 day(s) = 11 %

Method: other: static test according to Zahn-Wellens test method (later adapted as OECD Guideline 302B)
GLP: no
Test substance: other TS: mucochloric acid; purity not indicated

Result: Percentage values given under "Kinetic" refer to DOC elimination.
Kinetics indicative of DOC increase between days 0-8 (negative DOC elimination rates) and days 29-39 (sudden decrease) probably due to disintegration of sludge flocs.
Test condition: INOCULUM
- Source: industrial wastewater treatment plant (pre-adapted in a laboratory wwtp)
- Initial concentration: 1000 mg/L dry matter
TEST SYSTEM: closed vessels (Note: Woulfe bottle with inlet/outlet for aeration), no control of oxygen
TEST TEMPERATURE: 20-25 °C
ANALYTICAL PARAMETER: DOC (Note: TOC acc. to raw data, but this is equivalent to DOC because of the good solubility of MCA in water)
Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Restrictions: Documentation of experimental details confined to the above; no GLP study.
Flag: Critical study for SIDS endpoint
09-JUL-2004 (26)

Type: aerobic
Inoculum: activated sludge, industrial, adapted
Concentration: 200 mg/l related to DOC (Dissolved Organic Carbon)
748 mg/l related to Test substance
Degradation: 70 - 80 % after 45 day(s)
Kinetic:
17 day(s) = 4 %
21 day(s) = 15 %
28 day(s) = 55 %
39 day(s) = 68 %
45 day(s) = 76 %

Method: other: static test according to Zahn-Wellens test method (later adapted as OECD Guideline 302B)
GLP: no
Test substance: other TS: mucochloric acid; purity not indicated

Result: Percentage values given under "Kinetic" refer to DOC elimination.
Test condition: INOCULUM
- Source: from industrial wastewater treatment plant (pre-adapted in a laboratory wwtp)
- Initial concentration: 1000 mg/L dry matter
TEST SYSTEM: closed vessels (Note: Woulfe bottle with inlet/outlet for aeration), with control of oxygen
TEST TEMPERATURE: 20-25 °C
ANALYTICAL PARAMETER: DOC
Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions.
Restrictions: Documentation of experimental details confined to the above; no GLP study.
Flag: Critical study for SIDS endpoint
09-JUL-2004 (27)

Type: aerobic
Inoculum: other: activated sludge from laboratory waster water treatment plant
Degradation: < 10 % after 28 day(s)
Result: under test conditions no biodegradation observed

Method: other: Respirometric test
GLP: no
Test substance: other TS: mucochloric acid, purified technical grade

Method: The method is described in: Pagga U (1980) Respirometrischer Abbau- und Toxizitätstest mit Belebtschlamm zur Prüfung von Substanzen und Abwässern. Vom Wasser 55: 313-326
Result: <10% degradation based on BOD/COD or DOC elimination (on average)
Test condition: INOCULUM: Initial concentration 200 mg/L dry matter
TEST SYSTEM
- Culturing apparatus: respirometer
INITIAL TEST SUBSTANCE CONCENTRATION: 10/34/102/340/1023 mg/L (3/10/30/100/300 mg/L DOC)
DURATION OF THE TEST: 28 d
ANALYTICAL PARAMETER: BOD, DOC
Reliability: (3) invalid
Methodological deficiences: considerable divergences in the test vessels; only 2 considered for final test result; documentation unclear with respect to adaptation (yes/no) of inoculum

Type: aerobic
Inoculum: other: adapted activated sludge from laboratory waste water treatment plant
Contact time: 28 day(s)
Result: other: Mean BOD*100/COD after 28 days: ca. 75%; mean DOC elimination after 28 days: ca. 67%

Method: other: Respirometric test
GLP: no
Test substance: other TS: mucochloric acid, purified technical grade

Method: PRINCIPLE OF TEST METHOD:
Determination of the ultimate biodegradation of a test substance in a respirometric test. For the evaluation of biodegradation the relation between consumed oxygen (O2) and added carbon of the test substance (C) is calculated and expressed as O2/C-value. O2/C-values >2 indicate good biodegradability. In addition DOC (dissolved organic carbon) is measured at the end of the test and compared to the added test substance DOC. An elimination of >70% indicates good elimination. The chemical oxygen demand (COD) of the test substance can be compared to the BOD. A value >60% indicates good biodegradation.
The method is described in: Pagga U (1980) Respirometrischer Abbau- und Toxizitätstest mit Belebtschlamm zur Prüfung von Substanzen und Abwässern. Vom Wasser 55: 313-326

Result: DEGRADATION RATES AFTER 28 DAYS:
- BOD/COD percentage*):
54% at 102 mg/L TS corresponding to 30 mg/L DOC (addition of 120 mg/L DOC of synthetic sewage);
51% at 340 mg/L TS corresponding to 100 mg/L DOC (addition of 120 mg/L DOC of synthetic sewage);
113% at 102 mg/L TS corresponding to 30 mg/L DOC;
85% at 340 mg/L TS corresponding to 100 mg/L DOC.
Mean BOD*100/COD after 28 days: ca. 75%

- DOC elimination*):
73% at 102 mg/L TS corresponding to 30 mg/L DOC (addition of 120 mg/L DOC of synthetic sewage);
49% at 340 mg/L TS corresponding to 100 mg/L DOC (addition of 120 mg/L DOC of synthetic sewage);
77% at 102 mg/L TS corresponding to 30 mg/L DOC;
69% at 340 mg/L TS corresponding to 100 mg/L DOC.
Mean DOC elimination after 28 days: ca. 67%

KINETICS: no data

*) BOD*100/COD (0%) or DOC elimination (5%) at 1023 mg/L TS (300 mg/L DOC) not used for calculation of mean percentage because too high carbon

Test condition: INOCULUM:
- Initial concentration: 200 mg/L dry matter
TEST SYSTEM: respirometer
INITIAL TEST SUBSTANCE CONCENTRATION: 10/34/102/340/1023 mg/L (3/10/30/100/300 mg/L DOC)
DURATION OF TEST: 28 d
ANALYTICAL PARAMETER: BOD, DOC
MEDIUM

- mineral salt solution based on phosphate buffer
- Additional substrate: peptone and yeast extract
CONTROLS: (i) blank sample; (ii) substrate sample

Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test system and procedure)

09-JUL-2004 (29)

Type: aerobic
Inoculum: activated sludge, industrial
Concentration: 100 mg/l related to DOC (Dissolved Organic Carbon)
345 mg/l related to Test substance
Degradation: = 59 % after 87 day(s)
Kinetic:
12 day(s) = 10 %
26 day(s) = 1 %
28 day(s) = 12 %
37 day(s) = 0 %
87 day(s) = 59 %

Method: other: static test according to Zahn-Wellens test method (later adapted as OECD Guideline 302B)
GLP: no
Test substance: other TS: mucochloric acid; purity not indicated

Result: Percentage values given under "Kinetic" refer to DOC elimination.
Kinetics indicative of DOC increase at several time periods (negative or sudden drops of DOC elimination rates) probably due to disintegration of sludge flocs.
Test condition: INOCULUM
Source: from industrial wastewater treatment plant (adaptation not explicitly stated in reprint, but presumably not pre-adapted to mucochloric acid)
- Initial concentration: 1000 mg/L dry matter
TEST TEMPERATURE: 20-25 °C
ANALYTICAL PARAMETER: DOC

Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test system and procedure)

09-JUL-2004 (30)

Type: aerobic
Inoculum: activated sludge, industrial
Concentration: 400 mg/l related to DOC (Dissolved Organic Carbon)
1428 mg/l related to Test substance
Degradation: 80 - 90 % after 36 day(s)
Result: other: under test conditions elimination (primarily biodegradation) after lag phase, but not inherently biodegradable according to criteria of OECD Guideline 302 B
Kinetic:
16 day(s) = 10 %
21 day(s) = 10 %
27 day(s) = 24 %
29 day(s) = 46 %
34 day(s) = 84 %

Method: other: static test according to Zahn-Wellens test method (later adapted as OECD Guideline 302B)
GLP: no
Test substance: other TS: mucochloric acid; purity not indicated

Result: Percentage values given under "Kinetic" refer to DOC elimination.
Test condition: INOCULUM
Source: from industrial wastewater treatment plant (adaptation not explicitly stated in reprint, but presumably not pre-adapted to mucochloric acid)
- Initial concentration: 1000 mg/L dry matter
TEST TEMPERATURE: 20-25 °C
ANALYTICAL PARAMETER: DOC
Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test system and procedure)
09-JUL-2004 (31)

Type: aerobic
Inoculum: activated sludge, industrial
Concentration: 100 mg/l related to DOC (Dissolved Organic Carbon)
Contact time: 54 day(s)
Degradation: 80 - 90 % after 54 day(s)
Result: other: under test conditions elimination (primarily biodegradation) after lag phase, but not inherently biodegradable according to criteria of OECD Guideline 302 B
Kinetic:
28 day(s) =
42 day(s) = 3 %
48 day(s) = 50 %
49 day(s) = 51 %
50 day(s) = 80 %

Method: other: static test according to Zahn-Wellens test method (later adapted as OECD Guideline 302B)
GLP: no
Test substance: other TS: mucochloric acid; purity not indicated

Result: Percentage values given under "Kinetic" refer to DOC elimination.
Kinetics indicative of DOC increase between days 0-41 (negative TOC elimination rates, e.g. -24% at day 28; not entered in "Kinetic" field because only positive values allowed) probably due to disintegration of sludge flocs.
Test condition: INOCULUM
Source: from industrial wastewater treatment plant (adaptation not explicitly stated in reprint, but presumably not pre-adapted to mucochloric acid)
- Initial concentration: 1000 mg/L dry matter
TEST TEMPERATURE: 20-25 °C
ANALYTICAL PARAMETER: DOC
Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test system and procedure)
09-JUL-2004 (32)

Type: aerobic
Inoculum: activated sludge, industrial
Concentration: 100 mg/l related to DOC (Dissolved Organic Carbon)
368 mg/l related to Test substance
Degradation: 90 - 100 % after 35 day(s)
Result: other: under test conditions elimination (primarily biodegradation) after lag phase, but not inherently

biodegradable according to criteria of OECD Guideline 302 B

Kinetic:
13 day(s) = 0 %
20 day(s) = 51 %
22 day(s) = 4 %
25 day(s) = 16 %
28 day(s) = 62 %

Method: other: static test according to Zahn-Wellens test method (later adapted as OECD Guideline 302B)
GLP: no
Test substance: other TS: mucochloric acid; purity not indicated

Result: Percentage values given under "Kinetic" refer to DOC elimination.
Kinetics indicative of DOC increase between days 0-4 (negative DOC elimination rates) and days 20-28 (see data on kinetics) probably due to disintegration of sludge flocs.

Test condition: INOCULUM
Source: from industrial wastewater treatment plant (adaptation not explicitly stated in reprint, but presumably not pre-adapted to mucochloric acid)
- Initial concentration: 1000 mg/L dry matter
TEST TEMPERATURE: 20-25 °C
ANALYTICAL PARAMETER: DOC

Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test system and procedure)

09-JUL-2004 (33)

3.6 BOD5, COD or BOD5/COD Ratio

Method: other: according to DIN 38409 Part 51 (now DIN EN 5815-1)
GLP: no

C O D

Method: other: according to DIN 38409 Part 41
Year:
GLP: no
COD: = 543 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: < .004
Method:
Result: - BOD5 x 100/COD < 1%
- Evaluation of test result: No biodegradation under the conditions of the test

Test condition: INOCULUM: effluent from industrial (BASF) waste water treatment plant
Test substance: Mucochloric acid, purity not indicated
Reliability: (2) valid with restrictions
Test procedure in accordance with national standard methods with acceptable restrictions (Testing done 1981, but methods have not changed since then).
Restrictions: Documentation of experimental details confined to the above; no GLP study.
Flag: Critical study for SIDS endpoint

31-JUL-2002 (34)

3.7 Bioaccumulation

BCF: = 3.16

Method: other: calucalton Bcfwin, v 2.14

Result: log BCF = 0.50
Accepted calculation method
12-JUL-2004 (35)

3.8 Additional Remarks

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
LC50: = 123

Method: other: DIN 38412 Part 15
Year: 1982
GLP: no
Test substance: other TS: Mucochloric acid, technical grade, dried

Result: RESULTS: EXPOSED
- Effect data (Mortality after 96 hours):
From 10 fishes died 0 at 10, 21.5, 46.4 mg/L; 2 at 100 mg/L; 10 at 215 mg/L; 9 at 215 mg/L pH-adjusted
- Other effects: no symptoms at 10-46.4 mg/L; apathy, narcotic-like state, tumbling at 100 and 215 mg/L
RESULTS: CONTROL
- Number/percentage of animals showing adverse effects: 0

- LC50 CALCULATION: In accordance with the EU guideline used. The LC50 value was determined by means of a graphical estimation method based on the probit procedure because the steep concentration-effect courve precludes the use of a standard calculation method.
Input parameters: LC0 (set as 1%)=46.4 mg/L; LC100 (set as 99%)=215 mg/L (without pH adjustment); LC90 =215 mg/L (with pH adjustment)
Output: LC50 = 123 / 136 mg/l (without / with pH adaptation)
Confidence limits cannot be given due to the graphical evaluation method.

Test condition: TEST ORGANISMS
- Strain: Golden variety
- Size: body length 7.7 cm; body weight 4.5 g
DILUTION WATER
- Source: reconstituted freshwater according to DIN 38 412, Part 11, Draft Sept. 1981; preparation from fully demineralized tap water
- Aeration: continuously with oil-free air
- Alkalinity: 0.8 mmol/L
- Hardness: 2.5 mmol/L
- pH: about 8.0
- Conductance: max. 10 micro mho
TEST SYSTEM
- Concentrations: (i) without pH adjustment: 0, 10, 21.5, 46.4, 100, 215 mg/L; (ii) with pH adjustment: 215 mg/L
- Exposure vessel type: all-glass aquaria (30x22x24 cm)
- Number of fish per test concentration: 10
- Test temperature: 20-21 °C

- Dissolved oxygen: 7.0-8.6 mg/L in all groups (1-96 hours)
- pH: 7.1-7.9 at concentrations of 10, 21.5 and 46.4 mg/L (1-96 hours); 6.9 at 100 mg/L (1 hour); 4.6 at 215 mg/L (1 hour); 7.7-7.8 at 215 mg/L (1-98 hours) after pH adjustment; 7.7-7.8 in control (1-96 hours)
- Photoperiod: 16 hours light, 8 hours darkness

- Loading: 4.3 g fish per L test water
- Feeding during test: withdrawal of food 1 day before test begin
TEST PARAMETER: mortality and symptoms

Test substance: purity > 93 % w/w

Reliability: (2) valid with restrictions
Test procedure in accordance with national standard methods (comparable to OECD Guideline 203) with acceptable restrictions.
Restrictions: Documentation of experimental details confined to the above; no GLP study.

Flag: Critical study for SIDS endpoint

12-JUL-2004 (36)

Type: static
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 3 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50 (hep.) : = 115
EC50 (gill) : = 595

Method: other: calcein fluoresence intensity
GLP: no
Test substance: other TS: Mucochloric acid (MCA), purchased from Aldrich-Chemie, Steinheim, Germany

Remark: EC50 for decrease in fluorescence intensity was estimated from the dose/response curve.
Calcein fluorescence intensity was measured according to Hauglan RP, Larison KD (1992) Handbook of Fluorescence Probes and Research Chemicals, 5th edition, Molecular Probes Inc., USA

Result:
Hepatocytes EC50 = 0.68 +/- 0.08 mM (MCA)
Gill epithelial cells EC50 = 3.52 +/- 0.92 mM (MCA)
endpoint = decrease in fluorescence intensity (% decrease compared to control)

Test condition: - cells incubated at 15 °C for 2.5 h in cell type specific buffer solution with MCA
- 5.25 µM Calcein AM (acetomethoxymethyl ester derivate) dissolved in DMSO (final concentration 0.5 %) added after 2.5 h
- afer 30 min fluorescence intensity measured with a fluorescence spectrophotometer (excitation 500 nm and emission 520 nm)

Reliability: (2) valid with restrictions
Test procedure in accordance with scientifically accepted

methods. Restrictions: Documentation of experimental details confined to the above; no GLP study.

12-JUL-2004 (37)

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: = 6.25
EC50: = 12.9

EC100: = 50

Method: Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
GLP: no
Test substance: other TS: Mucochloric acid, technical grade, dried

Method: according to OECD 202
Remark: pH was < 6 only in the highest treatment. At 100 mg/l and lower, pH was >6.5 at 0 h and >7.8 at 48 h. There is a clear concentration-response relationship within 6.25 to 50 mg/l.
Result: EC0, EC50 and EC100-values are given as nominal concentrations,
endpoint = immobilization
EC50: VB 95% = 10.92 - 15.34 mg/l
Results after 24 h:
EC0 = 12.5 mg/l
EC50 = 27.7 mg/l
EC100 = 50 mg/l
Test condition: - TEST WATER: pH-value: 7.8, water total hardness: 2.94 mmol/l, alkanility up to pH 4.3: 0,86 mmol/l, conductivity: 620 µS/cm, molar ratio Ca:Mg = 4:1, Na:K = 10:1
- ILLUMINATION: artifical light, day:night-rhythm = 16:8 hours
light intensity: 5 µE at a wave of 400 - 750 nm,
- TEST TEMPERATURE: 292.0 - 294.0 K,
- O2-CONTENT: > 2mg/l
- TEST VOLUME: 10 ml
- VOLUME/ANIMALS: 2 ml
- NUMBER OF ANIMALS/VESSEL: 5
- TOTAL NUMBER OF ANIMALS/CONCENTRATION: 20
- AGE OF ANIMALS: 2-24 hours
- AGE OF STOCK ANIMALS: 2 -4 weeks
- CKECK OF THE STUDY: visually after 0, 3, 6, 24 and 48 h
- CONCENTRATION RANGE: 3.125 - 200 mg/l
- pH VALUES AT 0 HOURS: 6.6-7.7 (3.125-100 mg/l), 4.8 (200 mg/l); at 48 hours: 7.9-8.3 (3.125-100 mg/l), 5 (200 mg/l)
Test substance: purity > 93 % w/w
Reliability: (2) valid with restrictions
Guideline study with acceptable restrictions.
Restrictions: no GLP study.

Flag: Critical study for SIDS endpoint
12-JUL-2004 (38)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: = 42

Method: other: modified OECD Standard Protocol
GLP: no
Test substance: other TS: Mucochloric acid (MCA), purchased from Aldrich-Chemie, Steinheim, Germany

Remark: EC50 calculated as estimates using regression analysis after linearization of the dose/response curve by logarithmic transformation of the concentrations
Result: endpoint: Immobilization
EC50 = 0.25 +/- 0.04 mM (MCA)

Test condition: - TEST WATER: Standard reference water (SRW):pH-value: 7.6, 2.4 mM NaHCO3, 0.15 mM K2SO4, 2.0 CaCl2, 0.01 mM KH2PO4
- ILLUMINATION: artifical light, day:night-rhythm = 12:1 hours
- TEST TEMPERATURE: 21 +/- 1°C,
- O2-CONTENT: > 2mg/l
- TEST VOLUME: 50 ml
- VOLUME/ANIMALS: 2.5 ml
- NUMBER OF ANIMALS/VESSEL: 2.5 ml
- TOTAL NUMBER OF ANIMALS/CONCENTRATION: 60
- AGE OF ANIMALS: < 24 hours
- CKECK OF THE STUDY: visually after 24
- CONCENTRATION tested: 5 (with 3 replicates)

Reliability: (2) valid with restrictions
Test procedure in accordance with national standard method with acceptable restrictions.
Restrictions: Documentation of experimental details confined to the above; no GLP study.

08-AUG-2003 (37)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: = 62
EC20 : = 65

Method: other: method corresponds in principle to DIN 38412, part 9, Determination of inhibitory effect on the cell multiplication (comparable to OECD 201)
GLP: no
Test substance: other TS: Mucochloric acid, purity not indicated

Remark: The acidity of the substance might have influenced the toxicity.

Result: The EC values were calculated (linear regression analysis) from the concentration-response relationship.
The EC50 (72 h) value of 62 mg/L refers to biomass (EbC50) and is given as nominal concentration.
ErC50 were recalculated
72 h: 64.6
96 h: 49.4 mg/l.
The ErC10 values are the following:
72 h: 35.7
96 h: 16.1 mg/l.

Test condition: - Test strain: Scenedesmus subspicatus CHOD.
- Test type: static
- Inoculum density: about 10000 cells/ml
- Duration of test: 96 hours
- Test vessel: Erlenmeyer flaks (nominal volume 250 ml)
- Test volume: 10 ml
- Test concentrations: 7.81 - 125 mg/l
- Test conditions: temperature 293 K; initial pH 8.2 (measured after 0, and 96 h); illumination: artificial light
- 10 000 cells/ml,
- permanent illumination; light intensity: 120 µE/m2s
- Parameter: fluorometric determination of biomass after 24, 48, 72 and 96h (linearity between fluorometric values and cell counts was verified).

Reliability: (2) valid with restrictions
Test procedure in accordance with national standard method (comparable to OECD Guideline 201) with acceptable restrictions.
Restrictions: Documentation of experimental details confined to the above; no GLP study
Flag: Critical study for SIDS endpoint
26-JUL-2004 (39)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 17 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC10: = 3.6
EC50: = 6.4
EC90 : = 11.6

Method: other: DIN 38412, part 8, Determination of the inhibitory effect on the cell multiplication
GLP: no
Test substance: other TS: Mucochloric acid, purity not indicated

Result: The EC50 values given are nominal concentrations.
The acidity of the test substance might have influenced the toxic effects (pH values in uninoculated cultures at 0 and 17 hours: about 7; in inoculated cultures at 17 hours: 4.3-6.3).
Test condition: The test strain of Pseudomonas putida DSM 50026 used is obtained in regular intervals from DSM.
Duration of the test: 17 hours,
temperature during the test: 20 °C,
preculture: 100 ml, test culture: 10 ml.
The test substance was tested in the concentration range between 0.39 and 100 mg/l (nominal).
Reliability: (2) valid with restrictions
Test procedure in accordance with national standard method with acceptable restrictions.
Restrictions: Documentation of experimental details confined to the above; no GLP study.
Flag: Critical study for SIDS endpoint
28-JUL-2002 (40)

Type: aquatic
Species: other bacteria: BASF activated sludge
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 2000
EC20 : = 700

Method: OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
GLP: no
Test substance: other TS: mucochloric acid; purity not indicated

Test condition: INOCULUM: activated sludge from industrial (BASF) wwtp
CONCENTRATION OF ACTIVATED SLUDGE: 1000 mg/L dry matter
RESPIRATION IN THE BLANK: 11 mg/L*h after 30 min

Reliability: (2) valid with restrictions
Guideline study with acceptable restrictions.
Restrictions: Documentation of experimental details confined to the above; no GLP study.

29-JUL-2002 (41)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

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

In Vitro/in vivo: In vivo
Type: Absorption
Species: rat
Vehicle: other: traganth suspension
Route of administration: dermal
Exposure time: 1 hour(s)

Method: other: percutaneous absorbtion for details see freetext
Year: 1961
GLP: no
Test substance: other TS: Mucochloric acid, pure

Result:
MORTALITY:
- 2 animals of 5 died within 1 resp. 2 hours after end of exposure

SYSTEMICAL EFFECTS:
- No clinical effects observed on animals that died
- Apathy in surviving animals up to day 2

LOCAL EFFECTS:
- After application slight erythema and edemo on abdominal skin
- After 3 days yellow scaling resp. anemical superficial crust formation
- After 10 days crusts fell off, in one animal scar formation

PATHOLOGY:
- Organs gross necropsy findings
- In 1 animal gelatineous altered tissue in the area of application

Test condition:
ANIMALS:
- number of animals: 5
- strain: not specified
- sex: male

TEST SUBSTANCE ADMINISTRATION.
- concentration: 30% traganth suspension of MCA
- application volume: 2 ml
- application area approximately: 30 cm^2
- calculated dose: 600 mg/animal; approx. 3000 mg/kg bw
- exposure time: 1 hour

TEST PROCEDURE:
- abdominal fur shaven prior to application
- animals fixed in a special bath tub filled with 2 ml of test substance preparation
- after end of application period washing of the skin with Lutrol 9 and drying with cellulose
- observation period: 3 weeks

Reliability: (3) invalid
Not accepted study method; not in accordance to guidelines; due to the fixation process animals may be stressed; high dose applied; corrosive test substance concentration applied

07-JUL-2003 (42)

In Vitro/in vivo: In vivo
Type: Absorption
Species: rat
Vehicle: water
Route of administration: dermal
Exposure time: 1 hour(s)

Method: other: percutaneous absorbtion for details see freetext
Year: 1961
GLP: no
Test substance: other TS: Mucochloric acid (pure

Result: MORTALITY:
- 1 animals of 5 died on day 5 after exposure; no gross macroscopic evaluation possible because of kanibalism

SYSTEMICAL EFFECTS:
- Apathy from 4 hours after application up to day 2

LOCAL EFFECTS:
- in one animal petechial bleeding; edema and erythema 3 hours after application
24 hours after application skin partly pargement like crust formation
- 5 days after application circumscribed crusts
- After 12 days crusts fell off
- 3 weeks after application increased hair growth

PATHOLOGY:
- No gross macroscopic observations in surviving animals

Test condition: ANIMALS:
- number of animals: 5
- strain: not specified
- sex: 5 males

TEST SUBSTANCE ADMINISTRATION.
- concentration: 30% traganth suspension of MCA
- application volume: 2 ml
- application area approx. 30 cm^2
- calculated dose: 600 mg/animal; approx 3,000 mg/kg bw
- exposure time: 1 hour

TEST PROCEDURE:
- abdominal fur shaven 5 days prior to application
- animals fixed in a special bath tub filled with 2 ml of test substance preparation
- after end of application period washing of the skin with Lutrol 9 and drying with cellulose
- observation period: 3 weeks

Reliability: (3) invalid
Not accepted study method; not in accordance to guidelines; due to the fixation process animals may be stressed; high dose applied; corrosive test substance concentration applied

03-JUL-2003 (42)

In Vitro/in vivo: In vivo
Type: Absorption
Species: guinea pig
Vehicle: other: 20% solution of acetone : corn oil (9 : 1)

Route of administration: dermal

Method: other: percutaneous absorbtion for details see freetext
Year: 1950
GLP: no
Test substance: no data

Result: MORTALITY:
- all animals died

Test condition: TEST PROCEDURE:
- Test substance held in contact with depilated skin; no information given on exposure time
- Lowest dose level applied: 1000 mg/kg bw

Reliability: (3) invalid
limited information on test system and procedure; corrosive concentration applied

15-JUL-2003 (43)

In Vitro/in vivo: In vivo
Type: Absorption
Species: guinea pig
Vehicle: other: 5% solution of acetone : corn oil (9 : 1)
Route of administration: dermal

Method: other: percutaneous absorbtion for details see freetext
Year: 1950
GLP: no
Test substance: no data

Result: MORTALITY:
- all animals died within 2 days

LOCAL EFFECTS:
- skin edematous, thickened and necrotic

Test condition: TEST PROCEDURE:
- Test substance held in contact with depilated skin; no information given on exposure time
- Skin covered
- Lowest dose level applied: 250 mg/kg bw

Reliability: (3) invalid
limited information on test system and procedure; corrosive concentration applied

15-JUL-2003 (43)

In Vitro/in vivo: In vivo
Type: Absorption
Species: guinea pig
Vehicle: other: 5% acetone solution
Route of administration: dermal

Method: other: percutaneous absorption for details see freetext
Year: 1950
GLP: no

Test substance: no data

Result: MORTALITY:
- No mortality

CLINICAL SYMPTOMS:
- Weight loss in the middle of the week; original weight was recovered before the end of the test

LOCAL EFFECTS:
- No skin irritation noted

Test condition: TEST PROCEDURE:
- test substance preparation was rubbed on the back of each guinea pig daily for five days
- skin not covered
- Dose: 25 mg/kg bw

Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test system and procedure); Since application area was not covered oral uptake can not be excluded

Flag: Critical study for SIDS endpoint

15-JUL-2003 (43)

In Vitro/in vivo: In vitro
Type: Toxicokinetics

Method: other: Reaction with Cysteine: Test of enatiomeric recognition; Mutagenicity of chiral MCA-cysteine adducts
Year: 1993
GLP: no data

Test substance: other TS: Mucochloric acid, puri

Result: Mutagenicity of MCA and adducts from MCA and MCA-cysteine adducts given as molar mutagenicity (4 resp. 3 experiments per substance):

MCA: 2,340: 2,050; 1,870; 1,810 revertants/µmol; mean 2,020 revertants/µmol corresponding to 13.8; 12.1; 11.1; 10.7 revertants/µg; mean 12.0 revertants/µg

MCA-(R)-(+)-cysteine: 3.92; 9.56; 3.13; 5.26 revertants/µmol; mean 5.47 revertants/µmol

MCA-(S)-(-)-cysteine: 3.96; 6.37; 4.19; 5.54 revertants/µmol; mean 5.02 revertants/µmol

MCA-(R,S)-(+/-)-cysteine: 2.66; 4.83; 3.43 revertants/µmol; mean 3.64 revertants/µmol

Test condition: MUTAGENICITY ASSAY:
- Strain: TA100
- Standard plate incorporation assay
- Without metabolic activation
- Method according to Maron and Ames (1983) Mutat Res 113: 173-215
- Solvent: DMSO
- Three plates per dose level
- Zero dose: Solvent DMSO

- Controls (five plates per control): solvent control (DMSO); crystal violet; ampicillin; sodium azide
- Mutagenicity values as revertants per µg obtained from positive linear regression slopes of the ascending portion of the curve extending to the maximum value of revertants as determined by the statistical treatment of Bernstein et al. (1982) Mutat Res 97: 267-281.
- Statistical Significance: Difference of group means by t-test or ANOVA at the 95% level
- Calculation of the molar mutagenicity

CHIRAL TEST SUBSTANCES:
- MCA-(R)-(+)-cysteine; purity 99%
- MCA-(S)-(-)-cysteine; purity 99%
- MCA-(R,S)-(+/-)-cysteine; purity 99%

SPECTRA AND ELEMENTAL ANALYSES:
1H-NMR, 13C-NMR and 2D NMR:
- Brucker AMX 300 spectrometer
- 1H-NMR at 300 MHz, 13C-NMR at 75.45 MHz
- Chemical shift values relative to tetramethylsilane (TMS) (sigma = 0.00 ppm)
- Determination of quarternary CH, CH2 or CH3 carbons achieved by distorionless enhancement by polarization transfer (DEPT) experiments
UV-spectra:
- Variant DMS 100 spectrophotometer
EIMS:
- Finnigan 4021 mass spectrometer
Optical rotations:
- Perkin Elmer 141 polarimeter
- using a 10 cm path-length cell
Circular Dichroism (CD)
- Jasco Model ORD/UV5 modified for CD by Sproul Scientific part number SS-107
- determined in methanol solution
Elemental analysis
- performed by Desert Analytics

X-Ray Analysis
- X-ray structure of racemic form MCA-(R,S)-(+/-)-cystein determined by PJ Caroll (Chemistry Department University of Pennsylvania, Philadelphia)

Conclusion: Based on data of this study and on previous data (see LaLonde and Xie (1992) Chem Res Toxicol 5: 618-624)
MCA-(R)-(+)-cysteine is considered to be 2 to 4 times more mutagenic than MCA. No enantiospecific interaction between enantiomers and chiral DNA or enzymes involved in repair and replication could be concluded.

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments
Restrictions: Study not conducted in accordance with standard test guidelines or GLP

Flag: Critical study for SIDS endpoint

29-APR-2004 (44)

In Vitro/in vivo: In vitro
Type: Toxicokinetics

Method: other: Reaction with N-Acetylcystein
Year: 1992
GLP: no data
Test substance: other TS: Mucochloric acid, puri

Result: Inactivation of mutagenicity by reaction of MCA with NCA:
- after 6 h only 53% of initial mutagenicity
Kinetics of inactivation:
-second order rate constant at initial concentration ratios of MCA/NCA 2:1, 1:1, 1:2
Products:
- three products that resulted from the displacement of chlorine from C-3 or C-4 of MCA determined in levels of 7 (product 4), 2 (product 5) and 0.3% (product 6a)
- mutagenicity of products: product 4: nonmutagenic; product 5: weakly mutagenic, product 9a (product with chlorine conservation): comparable to MCA or more mutagenic than MCA

Test condition: Chemicals:
(R)-(+)-N-Acetylcystein (NCA) from Aldrich
(R)-(+)-Cysteine from Aldrich

Chromatography:
TLC:
- Merck silica gel 60F-254 sheets
Flash chromatography
- Merck Kieselgel 60 (230-400 mesh)
HPLC:
- Shimadzu LC-6A
- Column: ODS column (4.6 x 150 mm)
- Elution: idiocratically by 35% MeOH-water at ambient temperature at a flow rate of 1 ml/min
- detector wave length: 254 nm

Spectra and Elemental analysis:
NMR:
- Brucker AMX 300 spectrometer
- Standard for chemical shift values: TMS
- Dissolved in CDCl3 solution
- Determination of CH3, CH2, CH or quaternary carbons by 13C off-resonance or fully coupled spectra or DEPT experiments
1H-NMR:
- 300 MHz
13C-NMR:
- 75.45 MHz
2D-NMR:
UV-spectra:
- Kontron Uvikon 860
IR-spectra:
- Perkin-Elmer 1310 spectrometer
- in CH2Cl2 solutions
Chemical ionisation ass spectrometry (CIMS) and Electron impact mass spectrometry (EIMS):
- Finnigan 4021 mass spectrometer
- Reagent gas for CIMS: methane
Fast atom bobardment-high-resolution mass spectrometry (FAB-HRMS):
- determined by the Midwest Center of Mass Spectrometry, Department of Chemistry, University of Nebraska, Lincoln, NE
Optical rotation:

- Perkin Elmer 141 polarimeter
Elemental analysis
- performed by Desert Analytics, Tucson, AZ

Kinetics:
- Mixture of 15 µl of 0.037 M solutions of MCA and 7.5, 15 or 30 µl of 0.037 M solution of NAC in 0.1 M phosphate buffer (pH 7)
- Incubation in 1.0 cm sample cuvette containing 2 ml of buffer
- Immediate dilution with 3.5 ml of buffer
- Incubation in: cuvette holder at 25 °C
- Reference cuvette contains 0.1 M phosphate buffer
- Measuring of absorbance over the range from 200-400 nm over a period of 6 h.
- Kinetic data determined by decreasing absorbance at 261 nm (MCA) and increasing absorbance at 311 nm

Reaction of MCA with NAC:
a) in buffered aqueous solution
- Mixture of 340 mg MCA and 320 mg NAC in 0.1 N phosphate buffer (50 ml of 0.1 M K2HPO4 and 31 ml of 0.1 M KH2PO4) (pH 7.0)
- Incubation: under nitrogen at 30-35 °C overnight
- Acidification to pH 2 with 10% HCl
- Extraction with Acetic acid
- Washing of extract with brine and drying over Na2SO4
- Evaporation of solvent
- Treatment with diazomethane in ether
- Chromatography of the crude product on silica gel with CH2Cl2-Methanol (98:2)
- Characterization of the determined products
b) in acetone solution
- Mixture of 510 mg MCA, 480 mg NAC, and 600 mg KHCO3 in 70 ml acetone
- Incubation: Stirring under nitrogen at 30-35 °C overnight
- Acidification to pH 2 with 10% HCl
- Extraction with Acetic acid
- Washing of extract with brine and drying over Na2SO4
- Evaporation of solvent
- Treatment with diazomethane in ether
- Chromatography of the crude product on silica gel with CH2Cl2-Methanol (98:2)
- Characterization of the determined products

Determination of Mutagenicity
a) of Inactivation reaction:
- Incubation of 2 ml 0.1 M phosphate buffer solution (pH 7) containing 9.60 x 10E-4 mM MCA and 9.60 x 10E-4 mM NCA at 37 °C
- Taking of aliquots (200 µl) after 0.5, 1, 2, 4 and 6 h after mixing and immediately freezing
- Control: Incubation of 2 ml 0.1 M phophate buffer (pH 7) with 9.60 x 10E-4 mM MCA at 37 °C
- Taking of aliquots (200 µl) after 0, 0.5, 1, 2, 4 and 6 h after mixing and immediately freezing
- Storage at -4 °C until performance of mutagenicity assay
- Testing of 3 50 µl aliquots per sample in parallel
b) of isolated compounds:
- MCA and the products 4, 5, and 9a were added in freshly

prepared DMSO solution to the top agar
Ames test:
- Standard plate incorporation Ames test with tester strain Salmonella typhimurium TA 100 without S9-Mix
- Solvent: DMSO
- 3 plates per test concentration except zero dose level (solvent) where 5 plates were tested
- Controls: negative control (solvent); crystal violet; ampicillin; sodium azid
- Recording of mutagenicity as revertants/plate versus time
- Calculation of revertants/µg from linear portion of the dose response plots; calculation molar mutagenicity (revertants/nmol) thereof

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP.

Flag: Critical study for SIDS endpoint

29-APR-2004 (45)

In Vitro/in vivo: In vitro
Type: Toxicokinetics

Method: other: Reaction with Glutathion
Year: 1994
GLP: no data
Test substance: other TS: Mucochloric acid, puri

Result: Reaction of MCA with Glutathion
- Components eluted in HPLC at 4.52, 6.74, 7.11 and 28.17 min retention time; in control experiment were peaks at 4.52, 6.74, 7.11 retention time absent
- peak at 4.52 min retention time = GSSG: 10.5% (1H NMR)
- peak at 6.74 and 7.11 min retention time GSH-MCA conjugates: 69.7% (HPLC)
- peak at 28.17 min retention time = MCA: 21.6% (HPLC)

- Discovery of GSSG as reaction product indicates an oxidation of GSH by MCA
- Reaction of MCA with GSH is accompanied by the formation of a radical species of MCA (EPR-analysis)
- it is unclear wether the MCA radical represents an intermediate leading eventually to nonmutagenic conjugates or whether it is involved in a separate shunt oxidation process depleting the reaction system of GSH that would otherwise be availabale for more efficient inactivation through the complete conjugation

Test condition: NMR Spectra and Chromatography:
1H NMR:
- in D20 at 300 MHz
- on a Bruker AMX 300 spectrometer
- Chemical shift values relative to TMS (sigma = 0.00 ppm)
HPLC:
- Shimadzu LC-6A
- at ambient temperature
- Column: Shimadzu ODS (150 x 4.6 mm)
- Isocratic elution with CH3CN/THF/H20 9:1:1 (pH 2.96)
- Flow rate 0.3 ml/min

- Detection wavelength: 254 nm

Reaction of MCA with GSH
- Mixture of 80 mg, 0.48 mmol MCA and 150 mg, 0.48 mmol GSH in 15 ml aqueous 0.1 M phophate buffer solution (K_2HPO_4/KH_2PO_4) at pH 7.0; buffer degassed for 6 h with a stream of N_2
- Incubation for 24 h under N_2
- Withdrawel of 1 µl portions with a syringe for HPLC analysis
- Component separation by eluent freeze-drying
- Dissolvance of powder in D_2O for H NMR analysis
- Control experiment: same conditions but without MCA

EPR
- About 10 ml of 0.1 M sodium phosphate buffer (pH 7) was purged with N_2 for at least 1 h
- Preparation of spin trap solution: Stirring 0.023M 2-methyl-2-nitrosopopane (tNB) in N_2-purged buffer at 35 °C for 2 h
- Addition of MCA (0.032 M) and glutathion (0.030 M)
- Incubation under stirring in closed containers for 20 h at room temperature
- EPR-spectrometer: Bruker ESP300
- Recording of spectra at 9.77 GHz wit 100-kHz modulation frequency
- Each incubation sample was either pipetted or apirated into a quartz flat cell centerd in an ER-4103 TM110 cavity
- Calibration of g-values of the radical adducts with a standard signal form Fremy's salt (g = 2.0057 +/- 0.0001)
- Computer simulation by laboratory intern software

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; no guideline study; in vitro results

Flag: Critical study for SIDS endpoint

29-APR-2004 (46)

In Vitro/in vivo: In vitro
Type: Toxicokinetics

Method: other: Reaction with Glutathion
Year: 1993
GLP: no data
Test substance: other TS: Mucochloric acid, puri

Result: REACTION PRODUCTS OF MCA WITH GSH:
- Formation of a mixture of two diastomers resulting from displacement of the C-4-Cl by the sulfur of GSH
- Ratio of diastomers: 1.5:1
- These two diasomers accounted for 70% of the product as determined by HPLC
- after recristalization the diastomeric product was 99% pure
- reaction of MCA with GSH without undergoing ring-chain tautomerism

Kinetics of MCA-GSH adduct formation at 25 °C:
- second order kinetcs for all three ratios tested MCA:GSH 1:1; 2:1; 1:2
- compared to reaction of MCA with N-acetylcysteine reaction of MCA with GSH is 5-6 times more reactive

Mutagenicity of MCA and MCA-GSH adduct

MCA: 2,130; 2,710; 2,310; 1,030 revertants/µmol; mean 2,800 revertants/µmol corresponding to 12.6; 16.0; 13.7; 23.9 revertants/µg; mean 16.6 revertants/µg

MCA-GSH: at lowest dose tested (20 resp. 50 µg/plate) increase of 30-40 revertants/plate relative to spontaneous revertants but no dose-dependent increase

Test condition: NMR Spectra and Chromatography:
TLC:
- Merck silica gel 60FG-254 sheets
- Solvent systems:
1H NMR, 13C NMR and 2D NMR:
- 1H NMR in D20 at 300 MHz
- 13C NMR at 75.45 MHz
- on a Bruker AMX 300 spectrometer
- Chemical shift values relative to TMS (sigma = 0.00 ppm)
- Determination of quarternary CH, CH2 or CH3 carbons achieved by distorionless enhancement by polarization transfer (DEPT) experiments
HPLC:
- Shimadzu LC-6A
- at ambient temperature
- Column: Shimadzu ODS (150 x 4.6 mm)
- Isocratic elution with CH3CN/THF/H20 9:1:1 (pH 2.96)
- Flow rate 0.3 ml/min
- Detection wavelength: 254 nm
UV Spectra:
- Kontron UVIKON 860 spectrophotometer
Optic rotation:
- Perkin Elmer 141 polarimeter
Elemental analyses:
- Performed by Desert Analytics
X-Ray structure analysis:
- determined by PJ Carroll (Chemistry Department, University of Pennsylvania, Philadelphia)
Circular Dichroism (CD)
- Jasco Model ORD/UV5 modified for CD by Sproul Scientific part number SS-107
- determined in methanol solution

Kinetics:
- Mixture of 15 µl of 0.037 M solutions of MCA and 7.5, 15 or 30 µl of 0.037 M solution of GSH in 0.1 M phosphate buffer (pH 7)
- Incubation in 1.0 cm sample cuvette containing 2 ml of buffer
- Immediate dilution with 3.5 ml of buffer
- Incubation in: cuvette holder at 25 °C
- Reference cuvette contains 0.1 M phosphate buffer
- Measuring of absorbance over the range from 200-400 nm over a period of 6 h.
- Kinetic data determined by decreasing absorbance at 261 nm (MCA) and increasing absorbance at 311 nm

Reaction of MCA with GSH
- Mixture of 40 mg, 0.24 mmol MCA and 74 mg, 0.24 mmol GSH in 8 ml aqueous 0.1 M phophate buffer solution (K2HPO4/KH2PO4) at pH 7.0

- Incubation at 37 °C under N2 over night
- Thereafter acification of the solution with 10% aqueous HCl
- Freeze-drying of aqueous phase
- Recrystalization from methanol-water of the freeze-dry residue

DETERMINATION OF MUTAGENICITY:
- Ames test according to Maron and Ames (1985) Mutat Res 113: 173-215
- Standard plate incorporation assay
- Tester strain Salmonella typhimurium TA 100 without S9-Mix
- Testing of MCA and the reaction product dissolved in freshly prepared Me2SO4 solution added to the top agar
- Three plates per dose level
- Zero dose: Solvent Me2SO4 (five plates per control)
- Controls (five plates per control): solvent control (Me2SO4); crystal violet; ampicillin; sodium azide
- Mutagenicity values as revertants per µg obtained from positive linear regression slopes of the ascending portion of the curve extending to the maximum value of revertants as determined by the statistical treatment of Bernstein et al. (1982) Mutat Res 97: 267-281.
- Calculation of the molar mutagenicity

Conclusion: Loss of mutagenicity of MCA by GSH conjugation:
MCA-GSH not mutagenic in Ames test TA 100 tester strain
GSH is more reactive and more specific for reacting with the closed ring form of MCA (without ring-chain tautomerism)

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; no guideline study; in vitro results

Flag: Critical study for SIDS endpoint

29-APR-2004 (47)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: other: Schmitt-Fischer
Sex: male/female
Vehicle: other: traganth
Value: = 400 mg/kg bw

Method: other: Determination of the approximative median lethal dose
Year: 1960
GLP: no
Test substance: other TS: Mucochloric acid, washed, pure, purity: >= 90%

Result: Symptoms of atonia and ataxia
Test condition: - Number of animals: 5-10 per dose
- Post-exposure observation period: 8-14 days

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with

standard test guidelines or GLP; documentation of experimental details limited to the above.
Flag: Critical study for SIDS endpoint
07-JAN-2004 (48)

Type: LD50
Species: rat
Strain: other: Schmitt-Fischer
Vehicle: other: traganth
Value: = 360 mg/kg bw

Method: other: Determination of the approximative median lethal dose
Year: 1961
GLP: no
Test substance: other TS: Mucochloric acid (pure), neutralized with NaOH (pH approx. 6)

Result: Symptoms of atonia and ataxia
Test condition: - Number of animals: 5-10 per dose
- Preparation of test substance: neutralization with NaOH; pH of sodium salt solution of mucochloric acid: ca. 6
- Post-exposure observation period: 8-14 days
Conclusion: The toxicity (LD50 and symptoms) of the neutralized substance (sodium salt) is similar to that of the free acid.
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above.
Flag: Critical study for SIDS endpoint
07-JAN-2004 (42)

Type: LD50
Species: rat
Strain: other: US rats (inhouse breeding)
Sex: male/female
No. of Animals: 10
Vehicle: other: traganth-water solution
Value: = 300 mg/kg bw

Method: other: Determination of the approximative median lethal dose
Year: 1964
GLP: no
Test substance: other TS: Mucochloric acid, technical grade; contains 7-10% suds ("Mutterlauge")

Result: After application signs of atonia and ataxia; gross pathology after 7-day postexposure period showed no effects.
Test condition: - Post-exposure observation period: 7 days
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above.
Flag: Critical study for SIDS endpoint
07-JAN-2004 (49)

Type: LD50

Species: rat
Vehicle: other: 20% suspension in corn oil
Value: 50 - 100 mg/kg bw

Year: 1950
GLP: no
Test substance: no data

Result: MORTALITY:
- According to report, the oral LD50 was 50 - 100 mg/kg bw "killing rats given doses of as low as 100 mg/kg while all lower doses survived."
- "all death occured in a matter of hours"

SYMPTOMS:
Gasping and clonic convulsions were reported as symptoms in the higher dose groups.
Reliability: (4) not assignable
Documentation not sufficient for assessment (no other information given on test procedure, purity of test substance and results; unclear if all animals of the 100 mg/kg bw group died and if other groups were tested)
02-JUL-2003 (43)

Type: LD50
Species: rat
Strain: no data
Sex: no data
Vehicle: water
Value: = 190 mg/kg bw

Method: other: no information given
Year: 1971
GLP: no data
Test substance: no data

Result: The following signs and symptoms were reported:
- Clinical signs: excitation beginning 10-15 minutes after administration followed by reduced activity, laboured breathing and reduced breathing frequency
- Time to death: 1-3 days
Test condition: No further information on test conditions given
Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test procedure, no information on purity of test substance)
02-FEB-2004 (50)

Type: LD50
Species: rat
Value: = 294 mg/kg bw

Test substance: no data

Reliability: (4) not assignable
Documentation not sufficient for assessment (no other information given on test procedure and results)
17-JUL-2002 (51)

Type: LD50

Species: rat
Value: 100 mg/kg bw

Test substance: no data

Reliability: (4) not assignable
Secondary literature; no further information given
25-JUL-2002 (52)

Type: LD50
Species: rat
Value: 500 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS: Mucochloric acid (raw), purity as given in 1.1: >=90%

Test substance: Mucochloric acid, raw
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.
17-JUL-2002 (53)

Type: LD50
Species: rat
Value: 350

Method: other: BASF-Test
GLP: no
Test substance: other TS: Mucochloric acid (raw), purity as given in 1.1: >=90%, but neutralized with NaOH (pH approx. 6)

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.
17-JUL-2002 (42)

Type: other: Single dose application
Species: rat
Strain: no data
Sex: no data
Vehicle: water
Doses: 1/16 of LD50 = 12 mg/kg bw by gavage

Method: other: no data
Year: 1971
GLP: no data
Test substance: no data

Result: Histology 24 h after adminstration
- signs of irritation in the organs of the gastrointestinal tract,

- signs of irritation of the breathing organs
- circulatory disturbance and dystrophic changes of zentral nerveous system, liver, heart and kidney

Test condition: no further information on test conditions given
Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test procedure, no information on purity of test substance)
02-FEB-2004 (50)

Type: LD50
Species: mouse
Value: = 84 mg/kg bw

Test substance: no data

Reliability: (4) not assignable
Documentation not sufficient for assessment (no other information given on test procedure and results)
25-JUL-2002 (50)

Type: LD50
Species: mouse
Vehicle: other: 20% suspension in corn oil
Value: 200 - 400 mg/kg bw

Year: 1950
GLP: no
Test substance: no data

Reliability: (4) not assignable
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above
28-JAN-2004 (43)

Type: LD50
Species: mouse
Strain: no data
Sex: no data
Vehicle: water
Doses: no data
Value: = 54.5 mg/kg bw

Method: other: no data
Year: 1971
GLP: no data
Test substance: no data

Result: The following signs and symptoms were reported:
- Clinical signs: excitation beginning 10-15 minutes after administration followed by reduced activity, laboured breathing and reduced breathing frequency
- Time to death: 1-3 days

Test condition: no further information on test conditions given
Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test procedure, no information on purity of

test substance)
02-FEB-2004 (50)

Type: LD50
Species: rabbit
Value: 160 mg/kg bw

Test substance: no data

Reliability: (4) not assignable
Secondary literature; no further information given
25-JUL-2002 (52)

Type: LD50
Species: guinea pig
Value: 100 mg/kg bw

Test substance: no data

Reliability: (4) not assignable
Secondary literature; no further information given
28-JAN-2004 (52)

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Strain: Sprague-Dawley
Sex: male/female
No. of Animals: 10
Exposure time: 4 hour(s)
Value: > 5.1 mg/l

Method: other: dynamic inhalation test with head-nose only exposure and analytical monitoring
Year: 1980
GLP: no
Test substance: other TS: Mucochloric acid, technical grade, dried, purity >98%

Result: MORTALITY: no deaths during exposure or 14-day post-exposure period
CLINICAL SIGNS: escape attempts, preening, dyspnea and salivation during exposure; no symptoms 13 days after exposure
BODY WEIGHT: no effect on absolute b.w. after 7 and 14 days; relative b.w. gain significantly reduced in males after 7 and 14 days, slightly reduced in females after 7 days
NECROPSY FINDINGS: no effects
Test condition: TEST ORGANISMS:
- Weight at study initiation: 185 +/- 15 g
ADMINISTRATION:
- Type of exposure: head-nose only
- Concentrations: only one exposure group with a nominal concentration of 17.6 mg/L corresponding to a mean measured concentration of 5.1 mg/L (8 measurements of between 4.9-5.3 mg/L)
- Particle size: mass median aerodynamic diameter: 4.57 µm +/- 2.21 (geometric st. deviation); impactor sampling, gravimetric determination

- Type or preparation of particles: dust aerosol
EXAMINATIONS: clinical examination and mortality daily; gross pathology after 14-day post-examination period

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
One dose level acceptable because no compound-related mortality at exposure concentration of approx. 5 mg/L.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP.

Flag: Critical study for SIDS endpoint

02-FEB-2004 (54)

Type: other: Russian non standard test - Determination of limit concentration
Species: rat
Strain: no data
Sex: no data
Doses: no data
Value: = 3.8 mg/m³

Method: other: no data
Year: 1971
GLP: no data
Test substance: no data

Test condition: - dust exposure
- Determination of the threshold concentration after single inhalation exposure by alteration of the summative threshold coefficient???
- Information on test method limited to the above

Reliability: (3) invalid
Methodological deficiences: no standard test method, no reliable analytical determination of test-substance concentration; results in obvious discrepancy to other studies

02-FEB-2004 (50)

Type: other: inhalation hazard test
Species: rat
Exposure time: 8 hour(s)

Method: other: BASF-Test
Year: 1964
GLP: no
Test substance: other TS: Mucochloric acid, technical grade; contains 7-10% suds ("Mutterlauge")

Remark: No mortality and no symptoms in all 12 rats exposed for 8 hours to an enriched and saturated atmosphere at 20 °C and observed for 7 days. No gross pathology findings.

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above

02-FEB-2004 (49)

Type: other: inhalation hazard test
Species: rat
Exposure time: 8 hour(s)

Method: other: BASF-Test
Year: 1964
GLP: no
Test substance: other TS: Mucochloric acid, washed, pure, purity: >= 90%

Remark: No mortality and no symptoms in all 6 rats exposed for 8 hours to an enriched and saturated atmosphere at 20 °C. No symptoms except for irritation of mucous membranes. No gross pathology findings.
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above
02-FEB-2004 (48)

Type: other: inhalation hazard test
Species: rat
Exposure time: 8 hour(s)

Method: other: BASF-Test
Year: 1960
GLP: no
Test substance: other TS: Mucochloric acid, washed, purity: >= 90%

Remark: No mortality and no symptoms in all 6 rats exposed for 8 hours to an enriched and saturated atmosphere at 100 °C. Symptoms: apathia. No gross pathology findings.
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above
02-FEB-2004 (53)

Type: other: inhalation hazard test
Species: rat
Exposure time: 8 hour(s)

Method: other: BASF-Test
Year: 1960
GLP: no
Test substance: other TS: Mucochloric acid (raw), purity as given in 1.1: >=90%

Remark: No mortality and no symptoms in all 6 rats exposed for 8 hours to an enriched and saturated atmosphere at 20 °C. No gross pathology findings.
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of

experimental details limited to the above
02-FEB-2004 (53)

Type: other: inhalation hazard test
Species: rat
Exposure time: 8 hour(s)

Method: other: BASF-Test
Year: 1960
GLP: no
Test substance: other TS: Mucochloric acid (raw), purity as given in 1.1: >=90%

Remark: No mortality and no symptoms in all 6 rats exposed for 8 hours to an enriched and saturated atmosphere at 100 °C. No gross pathology findings.
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above
02-FEB-2004 (53)

Type: other: Non standard method on determination of irritation threshold
Species: rabbit
Strain: no data
Sex: no data
Value: = 3.024 mg/m³

Method: other: no data
Year: 1971
GLP: no data
Test substance: no data

Test condition: - dust exposure
- parameter for irritation: breathing frequency
- information on test method limited to the above
Reliability: (3) invalid
Methodological deficiencies: no standard test method, no reliable analytical determination of test-substance concentration; results in obvious discrepancy to other studies
02-FEB-2004 (50)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Sex: male/female
No. of Animals: 5
Vehicle: water
Value: > 200 mg/kg bw

Method: other: in accordance with test guidelines of the US Department of Transportation (Fed.Reg. Paragraph 173.343)
GLP: no
Test substance: other TS: Mucochloric acid, technical grade, dry; purity > 98%

Result: No mortality; 1 rabbit showed slight erythema at site of application 96 hours after application.

Test condition: TEST ORGANISMS:
- Weight at study initiation: ca. 2-3 kg
- Number of animals: 5 males, 5 females

ADMINISTRATION:
- Area covered: ca. 7x7 cm = ca. 50 cm2; shaved dorsal and side area
- Occlusion: yes
- Vehicle: water
- Concentration in vehicle: 500 mg/ml
- Doses: 200 mg/kg bw (only dose applied)
- Duration of exposure: 24 hours

DURATION OF STUDY: 72 hours
EXAMINATIONS: mortality at 1, 24, 48 and 72 hours after application; systemic symptoms of intoxication and local irritative effects

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.

Flag: Critical study for SIDS endpoint

18-JUN-2003 (55)

Species: rat
No. of Animals: 5
Vehicle: other: traganth suspension

Method: other: BASF-Test
GLP: no
Test substance: other TS: Mucochloric acid (pure)

Remark: Percutaneous resorption test

Result: MORTALITY:
- 2 animals of 5 died within 1 resp. 2 hours after end of exposure

SYSTEMICAL EFFECTS:
- No clinical effects observed on animals that died
- Apathy in surviving animals up to day 2

LOCAL EFFECTS:
- After application slight erythema and edema on abdominal skin
- After 3 days yellow scaling resp. anemical superficial crust formation
- After 10 days crusts fell off, in one animal scar formation

PATHOLOGY:
- Organs gross necropsy findings
- In 1 animal gelatineous altered tissue in the area of application

Test condition: ANIMALS:
- number of animals: 5
- strain: not specified
- sex: male

TEST SUBSTANCE ADMINISTRATION.
- concentration: 30% traganth suspension of MCA
- application volume: 2 ml
- application area approximately: 30 cm²
- calculated dose: 600 mg/animal; approx. 3000 mg/kg bw
- exposure time: 1 hour

TEST PROCEDURE:
- abdominal fur shaven prior to application
- animals fixed in a special bath tub filled with 2 ml of test substance preparation
- after end of application period washing of the skin with Lutrol 9 and drying with cellulose
- observation period: 3 weeks

Reliability: (3) invalid
Not accepted study method; not in accordance to guidelines; due to the fixation process animals may be stressed; high dose applied; corrosive test substance concentration applied
28-JAN-2004 (42)

Species: rat
No. of Animals: 5
Vehicle: other: water

Method: other: BASF-Test
GLP: no
Test substance: other TS: Mucochloric acid (pure), neutralized with NaOH (pH approx. 6)

Remark: Percutaneous resorption test:
Result: MORTALITY:
- 1 animals of 5 died on day 5 after exposure; no gross macroscopic evaluation possible because of kanibalism

SYSTEMICAL EFFECTS:
- Apathy from 4 hours after application up to day 2

LOCAL EFFECTS:
- in one animal petechial bleeding; edema and erythema 3 hours after application
24 hours after application skin partly pargement like crust formation
- 5 days after application circumscribed crusts
- After 12 days crusts fell off
- 3 weeks after application increased hair growth

PATHOLOGY:
- No gross macroscopic observations in surviving animals

Test condition: ANIMALS:
- number of animals: 5
- strain: not specified
- sex: 5 males

TEST SUBSTANCE ADMINISTRATION.
- concentration: 30% traganth suspension of MCA
- application volume: 2 ml
- application area approx. 30 cm²
- calculated dose: 600 mg/animal; approx 3,000 mg/kg bw
- exposure time: 1 hour

TEST PROCEDURE:

- abdominal fur shaven 5 days prior to application
- animals fixed in a special bath tub filled with 2 ml of test substance preparation
- after end of application period washing of the skin with Lutrol 9 and drying with cellulose
- observation period: 3 weeks

Reliability: (3) invalid
Not accepted study method; not in accordance to guidelines; due to the fixation process animals may be stressed; high dose applied; corrosive test substance concentration applied

03-JUL-2003 (42)

Type: other: acute dermal toxicity test
Species: guinea pig

GLP: no
Test substance: no data

Result: Both 5 ml of a 20% and 5 ml of a 5% solution of the test substance in 9:1 acetone : corn oil was lethal to all animals (number not given) after occulusive administration to the depilated skin. This would indicate a LD50 of less than 250 mg/kg bw.
The skin of the treatet animals became edematous, thickened and necrotic.

Reliability: (4) not assignable
Documentation not sufficient for assessment (no other information given on test procedure, purity of test substance and results)

25-JUL-2002 (43)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: rat
Vehicle: other: 20% suspension in corn oil
Route of admin.: i.p.
Value: 10 - 25 mg/kg bw

Year: 1950
GLP: no
Test substance: no data

Result: MORTALITY:
- at 25 and 50 mg/kg bw delayed death 5 days after injection

SYMPTOMS:
- remarkable symptoms at higher doses: gasping and clonic convulsions

Test condition: Administration of a 20% suspension in corn oil
Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test procedure and results)

28-JAN-2004 (43)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: = 16 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS: Mucochloric acid, pure, neutralisized

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above; not a physiological route of application
28-JAN-2004 (42)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: = 16 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS: Mucochloric acid, washed, pure; purity >= 90%

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above; not a physiological route of application
29-APR-2004 (48)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: 12 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS: Mucochloric acid, raw

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above; not a physiological route of application
28-JAN-2004 (53)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: 18 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS: Mucochloric acid, raw, neutralized

Reliability: (2) valid with restrictions

Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above; not a physiological route of application

28-JAN-2004 (42)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: 20 mg/kg bw

Method: other: BASF-Test
GLP: no
Test substance: other TS: Mucochloric acid, technical

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above; not a physiological route of application

28-JAN-2004 (49)

Type: LD50
Species: mouse
Vehicle: other: 20% suspension in corn oil
Route of admin.: i.p.
Value: < 10 ml/kg bw

Year: 1950
GLP: no
Test substance: no data

Result: MORTALITY:
- Delayed death up to four days
Test condition: Administration of a 20% suspension in corn oil
Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test procedure and results)

02-JUL-2003 (43)

Type: other
Doses: 30 mg/kg or 6 mg/kg
Route of admin.: s.c.

Year: 1976

Result: Systemical effects described as:
"Marked decrease of plasma proteins: MP1 and MP2 acidic alpha mucoproteins; Marked increase in the levels of SGPt and LDH activities; most prominent changes observed on day 4 after administration"

Local effects described as:
"Local affection in the area of skin contamination"
Reliability: (3) invalid
No information on test method in english abstract; testing in

humans?; single administration assumed but not specified; not a physiological route of administration

29-APR-2004 (56)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration: other: 80% in water
Exposure: Occlusive
Exposure Time: 4 hour(s)
No. of Animals: 2
Vehicle: water
Result: corrosive

Method: other: in accordance with test guidelines of the US Department of Transportation, Paragraph 173.1200, Federal Register
Year: 1980
GLP: no
Test substance: other TS: Mucochloric acid, technical grade, dry; purity > 98%

Result: SCORE (average of both animals):
- Erythema: 3.5/4/4/4 after 4 hours/1/2/8 days; necrosis changing from soft to pergament-like
- Edema: 3/3/3/1.5 after 4 hours/1/2/8 days
OTHER EFFECTS: no systemic effects observed

Test condition: TEST ANIMALS:
- Strain: Vienna White
- Sex: male
- Body weigth: ca. 2.7 kg
ADMINISTRATION/EXPOSURE
- Total volume applied: 0.5 g/animal
- Area of exposure: ca. 2x2 cm
- Postexposure period: 8 days
- Removal of test substance: with mixture of water/lutrol (1:1) and subsequent plotting with cellulose
EXAMINATIONS
- Examined: local and systemic effects
- Scoring system: 1 - 4
- Examination time points: 4 hours and then each workday; last examination on day 8

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.

Flag: Critical study for SIDS endpoint

28-JAN-2004 (57)

Species: rabbit
Concentration: other: 50% in water
Exposure Time: 20 hour(s)
Vehicle: water
Result: corrosive

Method: other: according to principles of Draize Test

Year: 1964
GLP: no
Test substance: other TS: Mucochloric acid, highest purity grade

Result: SCORE
- Erythema: after 1 and 5 min. exposure: slight spotty redness with eschar formation; after 15 min. exposure: redness
- Edema: after 1 and 5 min. exposure: none; after 15 min. exposure: swelling
- Necrosis: scarring after 20 hours exposure

EVALUATION OF RESULTS: slightly irritating after 15 min. exposure; corrosive after 20 hours exposure

Test condition: TEST ANIMALS:
- Strain: Vienna White
- Number of animals: no data

ADMINISTRATION/EXPOSURE
- Area covered: shaved back
- Total volume applied: 0.5 g/animal
- Exposure duration: 1, 5, 15 min. and 20 hours
- Removal of test substance: (i) short-time test: removal with undiluted lutrol and subsequently with a mixture of water/lutrol (1:1), (ii) after 20-hour application: no removal

EXAMINATIONS
- Scoring system probably as with comparable BASF tests: (i) 0 (no irritation); (+) (slight erythema); + (well-defined erythema); (ii) well-defined; severe edema

Test substance: no additional information on purity of test substance available: purity probably > 98%

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.
Flag: Critical study for SIDS endpoint
05-JAN-2004 (58)

Species: rabbit
Concentration: other: 30% in water
Exposure Time: 20 hour(s)
Vehicle: water
Result: corrosive

Method: other: according to principles of Draize Test
Year: 1961
GLP: no
Test substance: other TS: Mucochloric acid washed (pure), purity >= 90%

Result: SCORE
- Erythema: after 1 min. exposure: slight; after 5 and 15 min. exposure: slight with eschar formation; after 20 hours exposure: well-defined with eschar formation
- Edema: after 1 min. exposure: none; after 5 and 15 min. exposure: well-defined edema; after 20 hours exposure: well-defined edema
- Necrosis: scarring after 20 hours exposure

EVALUATION OF RESULTS: irritating after 15 min. exposure; corrosive after 20 hours exposure

Test condition: TEST ANIMALS:
- Strain: Vienna White
- Number of animals: 2

ADMINISTRATION/EXPOSURE
- Preparation of test substance: 30 g pure mucochloric acid dissolved in Aq. dest. ad 100 ml (pH not reported; for comparison: pH 2.2 at 24 g/L; see 2.6.1)
- Area covered: shaved back (in addition ear with 20 hours application)
- Total volume applied: 0.5 g/animal
- Exposure duration: 1, 5, 15 min. and 20 hours
- Removal of test substance: (i) time test: removal with undiluted lutrol and subsequently with a mixture of water/lutrol (1:1), (ii) after 20-hour application: no removal

EXAMINATIONS
- Scoring system: (i) 0 (no irritation); (+) (slight erythema); + (well-defined erythema); (ii) well-defined; severe edema

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.

Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.

Flag: Critical study for SIDS endpoint

05-JAN-2004 (42)

Species: rabbit
Concentration: other: 30% in water, neutralized (see freetext Test conditions)
Exposure Time: 20 hour(s)
Vehicle: water
Result: corrosive

Method: other: according to principles of Draize Test
Year: 1961
GLP: no
Test substance: other TS: Mucochloric acid washed (pure), purity >= 90% neutralized

Result: SCORE
- Erythema: after 1 min. exposure: none; after 5 and 15 min. exposure: slight with eschar formation; after 20 hours exposure: well-defined with eschar formation
- Edema: after 1 and 5 min. exposure: none; after 15 min. exposure: well-defined edema; after 20 hours exposure: well-defined to severe (ear) edema
- Necrosis: after 20 hours

EVALUATION OF RESULTS: irritating after 15 min. exposure; corrosive after 20 hours exposure

Test condition: TEST ANIMALS:
- Number of animals: 2

ADMINISTRATION/EXPOSURE
- Neutralization of test substance: 30 g pure mucochloric acid dissolved in 14.92 g $NaHCO_3$ + Aq. dest. ad 100,0 (pH 7)

- Area covered: shaved back (in addition ear with 20 hours application)
- Total volume applied: 0.5 g/animal
- Exposure duration: 1, 5, 15 min. and 20 hours
- Removal of test substance: (i) time test: removal with undiluted lutrol and subsequently with a mixture of water/lutrol (1:1), (ii) after 20-hour application: no removal

EXAMINATIONS
- Scoring system: (i) 0 (no irritation); (+) (slight erythema); + (well-defined erythema); (ii) well-defined; severe edema

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.

Flag: Critical study for SIDS endpoint
07-JAN-2004 (42)

Species: rabbit
Concentration: 50 %
Vehicle: water
Result: corrosive

Method: other: BASF-Test
Year: 1960
GLP: no
Test substance: other TS: Mucochloric acid, washed, pure, purity >=90%

Result: Exposure period 1 min:
- Animal 1: slight erythema that was reversible 1 day after application;
- Animal 2: slight erythema from day 2 up to day 3; scaling from day 6 up to day 15

Exposure period 5 min:
- Animal 1: slight erythema up to day 3; scaling on day 8
- Animal 2: slight erythem up to day 3; scaling from day 6 to day 15

Exposure period 15 min:
- Animal 1: slight erythema up to day 10 and edema up to day 2; scaling from day 6 up to day 15
- Animal 2: erythema up to day 3 and slight edema up to day 1 partly extending the application area; scaling from day 6 to day 15

Exposure period 20 h, back:
- Animal 1: erythema, bleeding, edema, anemic areas, necrosis on day 6
- Animal 2: erythema, bleeding, edema, anemic areas, necrosis on day 6

Exposure period 20 h, ear:
- Animal 1: erythema, bleeding, edema, anemic areas, necrosis on day 6

- Animal 2: erythema, bleeding, edema, anemic areas, necrosis on day 2

Clinical symptoms:
- Animal 1: Apathy, lateral position, no respectively reduced food intake
- Animal 2: Apathy, reduced food intake, trembling of the hind legs

Test condition: TEST PROCEDURE:
- White vienna rabbits
- Application of a 50% aqueous paste
- 2 rabbits received the test substance preparation for 1, 5 and 15 minutes and for 20 hours to the shaved back and for 20 hours to the ear
- after the exposure for 1, 5 and 15 the skin was washed with Lutrol and with Lutrol:water (1:1)
- animals observed up to 2 month after application

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above.

05-JAN-2004 (48)

Species: rabbit
Concentration: 50 %
Exposure Time: 20 hour(s)
Vehicle: water
Result: corrosive

Method: other: BASF-Test
Year: 1964
GLP: no
Test substance: other TS: Mucochloric acid, technical grade; contains 7-10% suds ("Mutterlauge")

Result: Exposure period 1 min:
- distinct erythema and edema followed by degeneration of the superficial skin layers

Exposure period 5 min:
- anemic necrosis with bleeding and strong edema

Exposure period 15 min:
- anemic necrosis with bleeding and strong edema

Exposure period 20 h, back:
- Animal 1: erythema, bleeding, edema, anemic areas, necrosis on day 6
- Animal 2: erythema, bleeding, edema, anemic areas, necrosis on day 6

Exposure period 20 h, ear:
- Animal 1: erythema, bleeding, edema, anemic areas, necrosis on day 6
- Animal 2: erythema, bleeding, edema, anemic areas, necrosis on day 2

Clinical symptoms:
- Animal 1: Apathy, lateral position, no respectively reduced

food intake
- Animal 2: Apathy, reduced food intake, trembling of the hind legs

Test condition: TEST ANIMALS:

- Strain: Vienna White
- Number of animals: no data

ADMINISTRATION/EXPOSURE
- Area covered: shaved back
- Total volume applied: 0.5 g/animal
- Exposure duration: 1, 5, 15 min. and 20 hours
- Removal of test substance: (i) short-time test: removal with undiluted lutrol and subsequently with a mixture of water/lutrol (1:1), (ii) after 20-hour application: no removal

EXAMINATIONS
- Scoring system probably as with comparable BASF tests: (i) 0 (no irritation); (+) (slight erythema); + (well-defined erythema); (ii) well-defined; severe edema

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above.

07-JAN-2004 (58)

Species: rabbit
Result: irritating

Year: 1986
Test substance: no data

Reliability: (4) not assignable
Documentation not sufficient for assessment (no other information given on test procedure and results)

07-JAN-2004 (51)

Species: rabbit
Concentration: 50 %
Exposure Time: 20 hour(s)
Vehicle: water
Result: corrosive

Method: other: BASF-Test
Year: 1960
GLP: no
Test substance: other TS: Mucochloric acid (raw) (unpurified commercial grade)

Result: SCORE
1 min: slight erythema, slight edema with scaling and crust formation
5 min: slight erythema, slight edema and crust formation
15 min: well-defined erythema, slight edema and crust formation
20 hours: necrosis and well defined edema

EVALUATION OF RESULTS: irritating after 15 min. exposure; corrosive after 20 hours exposure

Test condition: TEST ANIMALS:

- Strain: Vienna White
- Number of animals: no data
ADMINISTRATION/EXPOSURE
- Area covered: shaved back shaved back (in addition ear with 20 hours application)
- Total volume applied: 0.5 g/animal
- Exposure duration: 1, 5, 15 min. and 20 hours
- Removal of test substance: (i) short-time test: removal with undiluted lutrol and subsequently with a mixture of water/lutrol (1:1), (ii) after 20-hour application: no removal
EXAMINATIONS
- Scoring system probably as with comparable BASF tests: (i) 0 (no irritation); (+) (slight erythema); + (well-defined erythema); (ii) well-defined; severe edema

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above.

07-JAN-2004 (53)

Species: rabbit
Concentration: 50 %
Exposure Time: 5 minute(s)
Vehicle: water
Result: corrosive

Method: other: BASF-Test
Year: 1964
GLP: no
Test substance: other TS: Mucochloric acid F technical grade, wet

Remark: Exposure time: 1 and 5 min.
Result: 1 min exposure:
- slight to well-defined erythema and severe edema, partly bleeding
- followed by scaling and/or necrosis

5 min exposure:
- severe erythema, bleeding and necrosis

Test condition: TEST ANIMALS:
- Strain: Vienna White
- Number of animals: no data
ADMINISTRATION/EXPOSURE
- Area covered: shaved back shaved back
- Total volume applied: 0.5 g/animal
- Exposure duration: 1, 5
- Removal of test substance: (i) short-time test: removal with undiluted lutrol and subsequently with a mixture of water/lutrol (1:1),
EXAMINATIONS
- Scoring system probably as with comparable BASF tests: (i) 0 (no irritation); (+) (slight erythema); + (well-defined erythema); (ii) well-defined; severe edema

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of

experimental details limited to the above.
07-JAN-2004 (59)

Species: rabbit
Concentration: 50 %
Exposure Time: 5 minute(s)
Vehicle: water
Result: corrosive

Method: other: BASF-Test
Year: 1964
GLP: no
Test substance: other TS: Mucochloric acid technical grade, 92-94 % purity

Remark: Exposure time: 1 and 5 min.
Result: 1 min exposure:
- slight to well-defined erythema and severe edema, partly bleeding
- followed by scaling and/or necrosis

5 min exposure:
- severe erythema, bleeding and necrosis

Test condition: TEST ANIMALS:
- Strain: Vienna White
- Number of animals: no data

ADMINISTRATION/EXPOSURE
- Area covered: shaved back
- Total volume applied: 0.5 g/animal
- Exposure duration: 1, 5 min
- Removal of test substance: (i) short-time test: removal with undiluted lutrol and subsequently with a mixture of water/lutrol (1:1),

EXAMINATIONS
- Scoring system probably as with comparable BASF tests: (i) 0 (no irritation); (+) (slight erythema); + (well-defined erythema); (ii) well-defined; severe edema

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above.
07-JAN-2004 (59)

Species: rabbit
Concentration: 50 %
Exposure Time: 5 minute(s)
Vehicle: water
Result: irritating

Method: other: BASF-Test
Year: 1964
GLP: no
Test substance: other TS: Mucochloric acid, highest purity grade

Remark: Exposure time: 1 and 5 min.
Result: 1 minutes:
slight spotted erythema followed by scaling
5 minutes:
slight spotted erythema followed by scaling

Test condition: TEST ANIMALS:
- Strain: Vienna White
- Number of animals: no data
ADMINISTRATION/EXPOSURE
- Area covered: shaved back shaved back
- Total volume applied: 0.5 g/animal
- Exposure duration: 1, 5
- Removal of test substance: (i) short-time test: removal with undiluted lutrol and subsequently with a mixture of water/lutrol (1:1),
EXAMINATIONS
- Scoring system probably as with comparable BASF tests: (i) 0 (no irritation); (+) (slight erythema); + (well-defined erythema); (ii) well-defined; severe edema

Test substance: no additional information on purity of test substance available: purity probably > 98%

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above.

07-JAN-2004 (59)

Species: guinea pig
Concentration: 5 %
Exposure Time: no data
Vehicle: other: solution in acetone : corn oil (9 : 1)
Result: corrosive

Year: 1950
GLP: no
Test substance: no data

Result: Skin edematous, thickened and necrotic
Test condition: TEST PROCEDURE:
- dose applied: 0.25 mg/kg
- skin covered

Reliability: (4) not assignable
Documentation not sufficient for assessment (no other information given on test procedure, purity of test substance and results)

07-JAN-2004 (43)

Species: guinea pig
Concentration: 5 %
Exposure Time: no data
Vehicle: other: solution in acetone
Result: not irritating

Year: 1950
GLP: no
Test substance: no data

Remark: very small dose applied
Test condition: TEST PROCEDURE:
- dose applied: 0.025 mg/kg
- skin not covered

Reliability: (4) not assignable

Documentation not sufficient for assessment (no other information given on test procedure, purity of test substance and results)

07-JAN-2004 (43)

5.2.2 Eye Irritation

Species: rabbit
Dose: 50 other: mg as a powder
Exposure Time: unspecified
Comment: not rinsed
No. of Animals: 2
Result: highly corrosive

Method: other: according to principles of Draize test
Year: 1964
GLP: no
Test substance: other TS: Mucochloric acid, highest purity grade;

Result: The most severe symptom was an opacity of the complete cornea area graded as opaque, being still present at day 8 (study termination). Such effects are not regarded to be reversible.
Test condition: In accordance with the principles of the Draize test
Test substance: no additional information on purity of test substance available: purity probably > 98%
Conclusion: Inflammatory and degenerative lesions of similar degree in both purest grade TS and technical grade TS.
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.
Flag: Critical study for SIDS endpoint
07-JAN-2004 (58)

Species: rabbit
Dose: 50 other: mg as powder
Exposure Time: unspecified
Comment: not rinsed
No. of Animals: 4
Result: corrosive

Method: other: according to principles of Draize test
Year: 1960
GLP: no
Test substance: other TS: Mucochloric acid, raw (unpurified commercial grade)

Result: The most severe symptom was a complete opacity of the cornea from the beginning throughout the study (observation time 2 weeks). Such effects are not regarded to be reversible.
Test condition: In accordance with the principles of the Draize test.
Observation time: 14 days
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of

experimental details limited.
Flag: Critical study for SIDS endpoint
29-APR-2004 (53)

Species: rabbit
Concentration: other: 30% in water
Exposure Time: unspecified
Comment: not rinsed
No. of Animals: 2
Vehicle: water
Result: highly irritating

Method: other: according to principles of Draize test
Year: 1961
GLP: no
Test substance: other TS: Mucochloric acid (pure),

Result: The most severe symptom was a complete opacity of the cornea from the beginning throughout the study (observation time 2 weeks). Such effects are not regarded to be reversible.
Test condition: One drop of a 30% suspension in water was placed in the conjunctival sac; scoring after 10 min., 1 and 24 hours, and 3, 8 and 14 days
Test substance: no additional purity on TS available
Conclusion: Inflammatory and degenerative lesions more distinct than with neutralized TS. According to authors, this is due to the fact that the TS applied in suspension is removed with the lacrimal fluid more slowly as compared to the neutralized and hence, solubilized TS.
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.
Flag: Critical study for SIDS endpoint
07-JAN-2004 (42)

Species: rabbit
Concentration: 30 other: % neutralized
Exposure Time: unspecified
Comment: not rinsed
No. of Animals: 2
Result: irritating

Method: other: according to principles of Draize test
Year: 1961
GLP: no
Test substance: other TS: Mucochloric acid (pure), neutralized

Result: The pH value at the time of testing was considered to be 6 (one hour value after preparation of a neutralized test substance of pH 7.1). It is noteworthy that all effects including slight corneal opacity were reversible at study termination (observation time: 2 weeks).
Test condition: ADMINISTRATION/EXPOSURE
- Preparation of test substance: 30 g pure mucochloric acid dissolved in 14.92 g NaHCO3 + Aq. dest. ad 100,0 (pH 7)
- Instillation: one drop was placed in the conjunctival sac; scoring after 10 min., 1 and 24 hours, and 3, 8 and 14 days
Test substance: no additional information on purity available

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.
Flag: Critical study for SIDS endpoint
07-JAN-2004 (42)

Species: rabbit
Dose: 50 other: mm^3 bulk volume as a powder
Exposure Time: unspecified
Comment: not rinsed
Result: corrosive

Method: other: BASF-Test
Year: 1960
GLP: no
Test substance: other TS: Mucochloric acid, washed, pure; purity >= 90 %

Result: 1 hour observation:
- edema, erythema, corneal opacity, corrosion
24 hour observation:
- edema, erythema, corneal opacity, corrosion
8 days:
- edema, erythema, corneal opacity, suppuration
Test substance: no additional purity information on TS available
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above.
29-APR-2004 (48)

Species: rabbit
Concentration: no data
Exposure Time: unspecified
Comment: no data
Vehicle: no data
Result: corrosive

Year: 1950
Test substance: no data

Result: Gross swelling, erythema and permanent damage to the conjunctiva and cornea was caused by
the instillation of "a small crystal" into the rabbit eye.
Reliability: (4) not assignable
Documentation not sufficient for assessment (no other information given on test procedure, purity of test substance and results)
07-JAN-2004 (43)

Species: rabbit
Result: irritating

Year: 1986
Test substance: no data

Reliability: (4) not assignable
Documentation not sufficient for assessment (no other information given on test procedure, purity of test substance and results)
07-JAN-2004 (51)

5.3 Sensitization

Type: Skin painting test
Species: guinea pig
Concentration 1st: Induction 10 % open epicutaneous
2nd: Challenge 1 % open epicutaneous
No. of Animals: 10
Vehicle: other: acetone
Result: not sensitizing

Method: other: open epicutaneous test
GLP: no
Test substance: other TS: highest purity grade (cristallized)

Result: SKIN REACTIONS FOLLOWING INDUCTION EXPOSURE: necrotic degeneration of skin with subsequent scarring
SKIN REACTIONS FOLLOWING CHALLENGE EXPOSURE: slight redness
EVALUATION OF RESULTS: Twelve hours after challenge of pretreated animals no differences in skin reactions observed as compared to control animals who were tested for primary irritation only.

Test condition: TEST ANIMALS:
- Number of animals in test group: 10
- Number of animals in control group: 3
ADMINISTRATION/EXPOSURE
- Concentration/dose used for induction: 10% in acetone/0.1 ml per application
- Induction schedule: Daily topical application of TS in vehicle to shaved skin on left flank 5 times a week for 2 weeks
- Resting period after induction: 10 days
- Concentration used for challenge: 1% in acetone
- Challenge schedule: TS applied topically once on right flank of animals
EXAMINATION ATFER CHALLENGE: 12 hours after application
OTHER: no further data reported on method/test conditions
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.
Flag: Critical study for SIDS endpoint
17-JAN-2002 (60)

Type: Skin painting test
Species: guinea pig
Concentration 1st: Induction 10 % open epicutaneous
2nd: Challenge 1 % open epicutaneous
No. of Animals: 10
Vehicle: other: acetone
Result: not sensitizing

Method: other: open epicutaneous test
GLP: no
Test substance: other TS: Mucochloric acid, technical grade; contains 7-10% suds ("Mutterlauge")

Remark: Test conditions and results are the same as reported in the test with the highest purity grade mucochloric acid.
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited.
Flag: Critical study for SIDS endpoint
17-JUL-2002 (60)

Type: no data
Species: other: human and experimental animals (see remarks)
Result: sensitizing

Test substance: no data

Remark: Mucochloric acid is described as "potent skin sensitizer in man and experimental animals". The handbook refers to unpublished, unavailable and not further specified data from Fasset DW, Laboratory of Industrial Medicine, Eastman Kodak Co., Rochester, N.Y.
Reliability: (4) not assignable
Manufacturer / producer data: unpublished with no details
Flag: Critical study for SIDS endpoint
25-JUL-2002 (61)

Type: Guinea pig maximization test
Species: guinea pig
No. of Animals: 5

Test substance: no data

Remark: 1/5 guinea pigs became sensitized; however, some reaction may have been obscured because of the high degree of irritation (no details on test conditions and results)
Reliability: (4) not assignable
Documentation not sufficient for assessment (no other information given on test procedure, test substance and results)
17-JUL-2002 (43)

Type: other: industrial health report
Species: human
Result: ambiguous

Test substance: no data

Remark: From 1955-1971 74 cases of occupational dermatoses caused by mucochloric acid and its by-products were registered in a chemical plant. Sensitization during the production of N-phenyl-dichloro pyrazinone was attributed to phenylhydrazine.
No further details of the cases were given.

Reliability: (4) not assignable
No details reported
17-JUL-2002 (62)

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: 14 days (day 6 to 19 post coitum; see freetext Test conditions)
Frequency of treatment: once daily
Doses: 5, 30 or 60 mg/kg bw/day
Control Group: yes, concurrent vehicle
NOAEL: = 5 mg/kg bw
LOAEL: = 30 mg/kg bw

Method: other: OECD Guide-line 414 "Teratogenicity" (see chapter 5.8.2)
Year: 2001
GLP: yes
Test substance: other TS: Mucochloric acid, techn. pure 99.3% (2x recrystallized); white, solid/crystalline

Result: TOXIC EFFECTS ON DAMS:
- Test groups: (1) 0 mg/kg bw/day; (2) 5 mg/kg bw/day; (3) 30 mg/kg bw/day; (4) 60 mg/kg bw/day

CLINICAL EXAMINATIONS:
- Mortality: no deaths in all test groups except for one incidental case on day 14 p.c. in group 4 (60 mg/kg bw/day).
- Clinical symptoms: no remarkable signs except for ptyalism in 24/25 females of 60 mg/kg bw/day group (day 13-17 p.c. until termination) indicating poor GI tolerance due to corrosive properties of test substance; loud breathing in 11/25 probably due to compensatory mechanism.
- Food consumption: significantly reduced (-8%) during first 3 days at 30 and 60 mg/kg bw/day, not statistically significantly reduced (-4%) on days 6-20.
- Body weight: at 30 and 60 mg/kg bw/day, clearly reduced during first 3 days(-23%; $p<0.05$ at 30 mg/kg bw/day; -14%, not statistically significant at 60 mg/kgbw/day).
- Corrected body weight gain: at 30 and 60 mg/kg bw/day, reduced (-13%; not statistically significant at 30 mg/kg bw/day; -17%, $p<0.05$ at 60 mg/kgbw/day), considered as treatment-related.
- Necropsy findings: no macroscopic findings except for whitish foci in the stomach of 4/25 (30 mg/kg bw/day) and 15/25 (60 mg/kg bw/day), respectively.

- Reproduction data: see chapter 5.8.2

Test condition: Teratogenicity study (see details in chap. 5.8.2)
Examinations being of relevance with respect to sub-acute effects on dams: mortality, body weight gain, corrected body weight gain (calculated after terminal sacrifice), food consumption, clinical symptoms;
Macroscopic post-mortem examination at termination on day

20:
principle thoracic and abdominal organs, particularly GI tract (stomach examined e.g. for signs of erosions/ ulcerations); gross examination of placentas

Reliability: (2) valid with restrictions
Guideline study, but not designed as repeated dose toxicity study; provides limited information on subacute toxicity

Flag: Critical study for SIDS endpoint

17-JUL-2002 (63)

Type: Chronic
Species: mouse **Sex:** male/female
Strain: other: hybrids B6C3F1 (C57BL/6xAKR)F1 and B6AKF1 (C57BL/6xC3H/Anf)F1
Route of administration: oral feed
Exposure period: 18 months
Frequency of treatment: continuously
Post exposure period: none
Doses: 56 ppm corresponding to ca. 7 mg/kg bw per day (see freetext for further details)
Control Group: other: (i) untreated animals; (ii) vehicle control; iii) 7 positive control groups

Method: other: carcinogenicity study (see chap. 5.7)
Year: 1986
GLP: no
Test substance: other TS: Mucochloric acid, not specified ("commercial source"; no further purification)

Result: MORTALITY: no significant effects compared to vehicle controls; mortalities (i) B6C3F1 mice: 2/18 treated vs. 2/18 control (male), 0/18 treated vs. 2/18 control (female); (ii) B6AKF1 mice: 1/18 treated vs. 0/18 control (male), 2/18 treated vs. 3/18 control (female)
CLINICAL SIGNS: not reported
BODY WEIGHT CHANGES: not reported for control animals
FOOD AND WATER CONSUMPTION CHANGES: not reported
NECROPSY FINDINGS: no significant incidences compared to untreated controls
COMMON OTHER LESIONS:
osteogenesis of spleen, hyperkeratosis of stomach: B6C3F1: 1/16 m (control: 3/16 m);
follicular hyperplasia - any site: B6C3F1: 3/16 m (control 1/16); B6AKF1: 2/16 f (control 0/15);
lymphoid infiltrate - any site: B6C3F1: 1/16 m (control 1/16); 2/18 f (control 0/16); B6AKF1: 2/17 m (control 0/18); 1/16 f (control 1/15);
fat necrosis - any site: B6C3F1: 1/18 f (control: not reported);
focal pneumonia: B6AKF1: 2/17 m; 4/16 f (control: aspiration pneumonia: B6C3F1: 3/16 m; 4/16 f; B6AKF1: 2/18 m; 3/15 f)

Test condition: This study was designed as carcinogenicity study (see chapter 5.7).
TEST ORGANISMS
- Age: 7 days
- Number of animals: 18 mice of each sex and each strain

ADMINISTRATION
(i) 21.5 mg/kg in 0.5% gelatin bw once daily by stomach

tube until age of 4 weeks (dose not readjusted according to body weigth gain), followed by (ii) ad libitum administration of 56 ppm TS, mixed into diet, after weaning until end of exposure period (corresponding to ca. 7 mg/kg bw per day)
Both doses were recorded as maximum tolerated doses by the authors, but no data available.

OBSERVATIONS AND FREQUENCY
Animals were observed daily for any abnormalities.

ORGANS EXAMINED AT NECROPSY:
- Macroscopic: thoracic and abdominal cavities
- Microscopic: major organs and all grossly visible lesions (thyroid gland not examined); following tumour groupings analyzed: hepatomas, pulmonary tumours, lymphomas, and total mice with tumours
OTHER EXAMINATIONS:
blood smears examined only in cases showing splenomegaly and lymphadenopathy;
no haematology, urinalysis, clinical chemistry, ophthalmoscopic examination, food and water consumption reported

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; only one dose applied; relevant parameters not examined.

Flag: Critical study for SIDS endpoint

12-JAN-2004 (64) (65)

Type: Sub-chronic
Species: rat **Sex:**
Strain: no data
Route of administration: inhalation
Exposure period: 5 months
Frequency of treatment: 5 hours/day
Post exposure period: no data
Doses: 0.42 mg/m3; 0.05 mg/m3 (0.00042 mg/l; 0.00005 mg/l)
Control Group: yes, concurrent no treatment

Year: 1971
Test substance: no data

Remark: 7 animals per treatment group; 6 animals in control group.
Changes in reflex behaviour, cholinesterase activity and number of leucocytes and monocytes were observed, which were not specific.
No details given on test conditions and results.

Result: - clincal observations, body weight, organ weights, functional status of the liver: no differences as compared to control group.

- variation in functional activity of the CNS: e.g. delayed lose of conditional adverse-effects reflex; in a test on continuance of a learned conditional reflex (Z. Ja. Lagno, 1968) a prolonged loose of the reflex (i.e. prolonged staying in the light part of the exposure chamber) was seen.

- Intermittently an increase of excitability combined with an increase in blood cholineesterase activity was noted.

Test condition: - 7 animals per treatment group, 6 animals per control group
- exposure in individual inhalation chambers
- exposure via an Sirokov atomizer
- no reliable analytical determination of dust concentration described
- clinical observation, body weight, organ weights (not specified), functional status of liver (not specified), funktional activity of CNS (not specified except conditional adverse-effects reflex)
- determination of blood choline-esterase

Reliability: (4) not assignable
Methodological deficiencies: no standard test method, no reliable analytical determination of test-substance concentration; results in obvious discrepancy to other studies

06-FEB-2004 (50)

Type: Sub-chronic
Species: rat **Sex:**
Strain: no data
Route of administration: oral unspecified
Exposure period: 4 months
Frequency of treatment: daily
Post exposure period: no data
Doses: 0.07 LD50 corresponding to ca. 13.3 mg/kg/d
Control Group: yes, concurrent no treatment

Method: other: no data
Year: 1971
GLP: no
Test substance: no data

Remark: No data on number of animals, strain, sex.
The dose administered did not cause any deaths during the study. Reported changes of behaviour and haematological and histological parameters were unspecific and did not significantly differ from control animals.

Result: - increased excitability (determined by summative threshold coefficient; SGK) after 1 and 1.5 month of exposure
- vital staining of the organs: decreased activity in spleen kidney aned lung.

Test condition: - body weight, weight of organs (not specified)
- blood analysis: hemoglobin, erythrocytes, leukocytes, differential white blood parameters, bromosulphalein probe, activity of the blood and serum cholinesterase
- histology of inner organs (not specified)

Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test
procedure and results; only one dose; only few parameters examined)

06-FEB-2004 (50)

Type: Sub-acute
Species: rat **Sex:**
Strain: no data
Route of administration: i.p.
Exposure period: 5 days

Frequency of treatment: 1 dose per day
Post exposure period: 4 days
Doses: 5 mg/kg bw/day
Control Group: no data specified

Year: 1950
GLP: no
Test substance: no data

Remark: Test substance applied as 5% suspension in corn oil to a "number of mature rats".
Symptoms: weight loss at an average of 40 g during the 5 days treatment period (regaining 20 g 4 days after last injection); decrease of average haemoglobin and red cell counts.
No details reported.

Result: BODY WEIGHT:
- Average weight loss per animal: 8 g/day
- Average weight loss per week: 40 g
- Regain of average 20 g weight four days after injection was stopped

HEMATOLOGY:
- Average hemoglobin: 3 g/animal
- Average red cell count: 1,810,000

Information on test result limited to the above

Test condition: Vehicle: Corn oil
Concentration: 5% suspension

CLINICAL PARAMETERS:
Determination of body weight

HEMATOLOGY:
Determination of hemoglobin and red cell counts at the end of exposure period but not at the end of the recovery period

Information on test conditions limited to the above

Reliability: (4) not assignable
Documentation not sufficient for assessment and methodological deficiences (limited information on test procedure and results, e.g. no data on purity of test substance, number of animals, no control group)

12-JAN-2004 (43)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
Concentration: 1.25 - 5000 µg/plate (with S-9 mix); 0.077 - 5000 µg/plate (without S-9 mix)
Cytotoxic Concentration: >=100 µg/plate (with); >=20 µg/plate (without metabolic activation)
Metabolic activation: with and without
Result: positive

Method: other: in accordance with Ames B.N. et al.: Proc.Nat.Acad.Sci USA, 70, 70, 2281-2285 (1973) and Ames B.N. et al.: Mut.Reg., 31, 347-364 (1975)

Year: 1981
GLP: no
Test substance: other TS: Mucochloric acid, technical grade, 99% purity

Remark: Formation of epsilonoA,A and epsiloncA,A probably by oxidative properties of MCA
Result: NUMBER OF REVERTANT COLONIES PER PLATE (multiple of control):
- With metabolic activation:
All test strains: no significant difference from control at 0.077-5000 µg/plate; bacteriotoxicity >= 100 µg/plate; positive controls valid

- Without metabolic activation:
TA 1535: 0; 1.7; 3; 4.5; 6; 10; 21; 16; 12 at 0.077; 0.155; 0.311; 0.625; 1.25; 2.5; 5; 10 µg/plate; bacteriotoxicity at 20/40/100/500/2500/5000 µg/plate
TA 1537 and TA 1538: no significant difference from control at 5-20 µg/plate; bacteriotoxicity at 40-5000 µg/plate
TA 98: 1.9; 2.3; 2.6; 2.5; 3.1 at 0.625; 1.25; 2.5; 5; 10 µg/plate; no significant difference from control at 0.077-0.311 µg/plate (this assay was not valid because positive control substance was negative)
TA 100: 0.1; 1.8; 2; 2.1; 2.7 at 0.077; 0.155; 0.311; 0.625; 1.25 µg/plate; 5; 8.3; 8.3; 7.2; 6.2 at 0.625; 1.25; 2.5; 5; 10 µg/plate; bacteriotoxicity at 20-5000 µg/plate
Positive controls valid except for TA 98 assay

EVALUATION OF RESULTS
- With metabolic activation: negative
- Without metabolic activation: Significantly positive with strains TA 1535, TA 98 and TA 100; negative with TA 1537 and TA 1538
Test condition: SYSTEM OF TESTING
- Type: plate incorporation assay
- Metabolic activation system: S-9 mix prepared from livers of male Sprague-Dawley rats induced by Aroclor 1254
SOLVENT: acetone
CONTROLS: negative (solvent) and positive controls:
- without metabolic activation: MNNG, 4-nitrophenylenediamine, 9-amino-acridinoiumchloridemonohydrate
- with metabolic activation: 2-aminoanthracene
- Incubation for 48 hours at 37°C
- Evaluation criteria:
a) doubling of the spontaneous mutation rate (control)
b) dose-response relationship
c) reproducability of the results
- no information on statistical methods available
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP.
Flag: Critical study for SIDS endpoint
12-JAN-2004 (66)

Type: Ames test
System of testing: Salmonella typhimurium TA 100
Concentration: 1.25 - 500 µg/plate (with S-9 mix); 1.25 - 10 µg/plate (without S-9 mix)
Cytotoxic Concentration: > 2.5 µg/plate (with); no cytotoxicity (without

metabolic activation)
Metabolic activation: with and without
Result: positive

Method: other: in accordance with Ames B.N. et al.: Proc.Nat.Acad.Sci USA, 70, 70, 2281-2285 (1973) and Ames B.N. et al.: Mut.Reg., 31, 347-364 (1975)
Year: 1985
GLP: no
Test substance: other TS: Mucochloric acid, technical grade, pure (twice crystallized); purity: ca. 99.9%

Result: NUMBER OF REVERTANT COLONIES PER PLATE (multiple of control; mean of triplicate results):
- With metabolic activation:
3.0; 2.8; 0.5; -; 0; 0; 0 at 1.25; 2.5; 5; 10; 20; 100; 500 µg/plate; bacteriotoxicity at > 2.5 µg/plate; positive control valid (factor 21 compared to control)

- Without metabolic activation:
2.2; 2.5; 3.2; 3.1 at 1.25; 2.5; 5; 10 µg/plate; positive control valid (factor 15 compared to control)

EVALUATION OF RESULTS
- With metabolic activation: positive at 1.25 and 2.5 µg/plate
- Without metabolic activation: positive from 1.25 - 10 µg/plate

Test condition: SYSTEM OF TESTING
- Type: plate incorporation assay
- Metabolic activation system: S-9 mix prepared from livers of male Sprague-Dawley rats induced by Aroclor 1254
- Number of plates: 3 test plates per dose or per control
SOLVENT: DMSO
CONTROLS: negative (solvent and sterility) controls; positive controls: 2-aminoanthracene (with S-9 mix), MNNG (without S-9 mix)
- Incubation for 48 hours at 37°C
- Evaluation criteria:
a) doubling of the spontaneous mutation rate (control)
b) dose-response relationship
c) reproducability of the results
- no information on statistical methods available

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP.
Flag: Critical study for SIDS endpoint
12-JAN-2004 (67)

Type: DNA damage and repair assay
System of testing: Escherichia coli K-12
Concentration: 0.04 - 10 µg/ml
Cytotoxic Concentration: no data
Metabolic activation: with and without
Result: positive

Method: other: according to Knasmueller S. and Mohn G.R.: Chem. Biol. Interact., 58, 109-116 (1986)
Year: 1994

GLP: no
Test substance: other TS: Mucochloric acid, purity min. 98%

Method: Liquid suspension assay
Result: RESULTS OF PRELIMINARY TEST (expressed as a) Mean number of uvrB/recA cells; b) absolute survival (%); c) Mean number of uvr+/rec+ cells; d) absolute survival (%); e) Relative % survival of uvrB/recA vs. uvr+/rec+):
0.00 µg/ml: a) 67+/-11; b) 100; c) 72+/-6; d) 100; e) 108
0.04 µg/ml: a) 67+/-10; b) 100; c) 66+/-5; d) 91; e) 108
0.36 µg/ml: a) 51+/-8; b) 76; c) 64+/-2; d) 88; e) 85
Pos. contr.: a) 6+/-1; b) 8; c) 29+/-6; d) 40; e) 22

RESULTS OF MAIN EXPERIMENTS (three plates per concentration):
No detailed data given; part of data derived from results figures
- Without metabolic activation: statistically significant concentration-dependent increase of relative survival used as parameter for genotoxic effects. Relative survival (%) at different concentrations (approximative values as taken from graph): 100; 112; 58; 25; 6 and 1% at 0; 0.1; 0.35; 1; 3 and 10 µg/ml, resp.

- With S9-mix: "almost complete loss of genotoxic activity" (no data given)
- With BSA: Reduction of genotoxic activity with increasing BSA concentration; relative survival (%) at different concentrations of BSA and 1 µg MCA/ml (approximative values as taken from graph): 24; 62; 96; 108% at 0; 5; 10 and 15 mg BSA/ml, resp.

Test condition: MEDIA: phosphate bufferes saline (PBS); neutral-red agar medium (NR-S agar); peptone-streptomycin boullion

METABOLIC ACTIVATION SYSTEMS:
- S-9 mix with liver homogenate from uninduced male mice
- Bovine serum albumine (BSA) added in different concentrations (0, 5, 10 or 15 mg/ml) to test the influence of BSA concentration on genotoxic activity of 1 µg MCA/ml

INDICATOR STRAINS: derived from E. coli K-12 343 113; strain 343/753 is uvrB/recA and lac+; strain 343/765 is uvr+/rec+ and lac-

PERFORMANCE OF TEST:
0.1ml aliquots of mixtures of the 2 strains transferred into Incubation tubes together with 0.8 ml PBS (replaced by S9-mix or BSA solutions in tests with metabolic activation) and 0.1 ml of aqueous solution of test substance. Incubation
for 120 min. at 37°C; at least 3 plates per incubation condition; dilution of mixtures and plating of aliquots; incubation of NR-S plates for 24 h at 37°C and 12 h at room temperature; counting of numbers of repair-deficient and repair-proficient colonies.

POSITIVE CONTROL: streptozotocin

EVALUATION:
Calculation of the differential survival frequencies on the basis of the ratio of the two strains in the control tubes

Statistics: One way analysis of variance
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP.
Flag: Critical study for SIDS endpoint
12-JAN-2004 (68)

Type: other: Host mediated assay
System of testing: Escherichia coli K-12
Concentration: see freetext
Cytotoxic Concentration: not applicable
Metabolic activation: without
Result: positive

Method: other: according to Knasmueller S.: Bull. Pol. Acad. Sci., 364, 225-234, (1988)
Year: 1994
GLP: no
Test substance: other TS: Mucochloric acid, purity min. 98%

Result: Repairable DNA damage was induced in E.coli bacteria recovered from all examined organs of mucochloric acid treated mice.

RESULTS OF EXPERIMENT 1 (dose: 200 mg/kg b.w.):
Mean relative survival rates (%) as calculated from 2 independent trials (all results for MCA are significantly different from negative control; P<0.01):
- stomach: 88+/-7 (control); 48+/-9 (MCA); 30+/-2 (positive control SZ); 60+/-4 (positive control IQ)
- lung: 101+/-10 (control); 61+/-7 (MCA); 20+/-2 (positive control SZ); positive control IQ not determined
- intestine: 85+/-7 (control); 37+/-7 (MCA); 24+/-3 (positive control SZ); 31+/-7 (positive control IQ)
- liver: 102+/-14 (control); 48+/-5 (MCA); 35+/-3 (positive control SZ); 21+/-3 (positive control IQ)
- kidney: 97+/-17 (control); 50+/-5 (MCA); 28+/-3 (positive control SZ); positive control IQ not determined
- spleen: 86+/-9 (control); 47+/-8 (MCA); 30+/-1 (positive control SZ); positive control IQ not determined

RESULTS OF EXPERIMENT 2 (dose: 40 mg/kg b.w.):
Only marginal, statistically not significant, effects in the different organs: relative survival rates >75%.
Test condition: MEDIA: phosphate bufferes saline (PBS); neutral-red agar medium (NR-S agar); peptone-streptomycin boullion

INDICATOR STRAINS: derived from E. coli K-12 343 113; strain 343/753 is uvrB/recA and lac+; strain 343/765 is uvr+/rec+ and lac-

PERFORMANCE OF TEST:
Escherichia coli K-12 strains were i.v. injected in mice; a single dose of the test substance was administered to mice by gavage; mice sacrificed after 2 hours. Induction of genotoxic effects in E. coli recovered from stomach and intestine (after removing contents and rinsing), lung, liver, kidney and spleen were investigated.

DOSES: 200 mg/kg b.w. (experiment 1) or 40 mg/kg b.w. (experiment 2) (2 independent trials in each experiment)

NUMBER OF ANIMALS IN EACH TRIAL:
- Experiment 1: 3 animals each for treatment group and negative control; 2 for each positive control
- Experiment 2: 6 animals for treatment group; no data on negative and positive control

POSITIVE CONTROLS: streptozotocin (SZ) or 2-amino-3-methyl imidazo[4,5-f]quinoline (IQ)

PREPARATION OF CELL SUSPENSIONS:
- liver, lung, kidneys and spleen transfered into tubes containing 3 ml of ice-cold PBS, homogenized and diluted
- stomach and intestine rinsed in sterile PBS after removal and subsequently transfered into tubes containing 3 ml of ice-cold PBS, homogenized and diluted

PLATE INCORPORATION:
- Incubation: 0.1 ml of the diluted and undiluted suspension
- Incubation for 24 h at 37°C, followed by 12 h at RT
- three plates per incubation condition

EVALUATION:
Calculation of survival frequencies in the organs on the basis of the ratio of the two strains in the injection mix:

DS (%) = Mx repair deficient / Mx repair proficient x F x 100
Mx = mean number of cells recovered from an organ at a given dose
Mc = mean number of cells in the incubation mix
F = MC repair proficient / Mc repair deficient

Statistics: Dunnets test

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP.

Flag: Critical study for SIDS endpoint

12-JAN-2004 (68)

Type: Mouse lymphoma assay
System of testing: L5178Y mouse lymphoma cells / TK locus
Concentration: 0.625 µg/ml - 10,0 µg/ml (with); 0.0313 µg/ml - 4.0 µg/ml (without metabolic activation)
Cytotoxic Concentration: 10 µg/ml (with); 0.5 and 1.0 µg/ml (without metabolic activation)
Metabolic activation: with and without
Result: positive

Method: other: in accordance with Clive, D. and Spector, J.F.S.: Mut.Res., 31, 17-29 (1975) (comparable to OECD 476)
Year: 1983
GLP: yes
Test substance: other TS: Mucochloric acid, technical pure; purity: >99%

Result: MUTANT FREQUENCIES (all values x10E-6):
- Without metabolic activation:
Small and not dose-dependent, but repeatable increases in mutant frequencies as follows:
0.0313 and 0.25 µg/ml: 29.3 and 35.0, resp., vs. minimum criterion of 29 (Trial 1)
1.0 µg/ml: 53.8 vs. minimum criterion of 29.2 (Trial 2)
0.5 µg/ml: 43.2 vs. minimum criterion of 33.6 (Trial 3)
(Results from trials 2 and 3 not reliable because of high toxicity observed at these concentrations, i.e. rel. growth 9.2% of control at 1 µg/ml and 15.6% at 0.5 µg/ml)
All negative controls in normal range; all positive controls valid

- With metabolic activation: two doses induced increases in mutant frequencies above the minimum criterion of 37.4, the highest dose induced a mutant frequency that was more than twice the minimum condition:
2.5 µg/ml: 40.2; 10.0 µg/ml: 102.9
All negative controls in normal range; all positive controls valid
Toxicity: relative growth rates 90.4, 109.7, 63.6, 76.2 and 17.4% at 0.625, 1.25, 2.5, 5 and 10 µg/ml, respectively

EVALUATION OF RESULTS
- Without metabolic activation: weakly mutagenic
- With metabolic activation: mutagenic
TS obviously converted into a less toxic, but more active form.

Test condition: METABOLIC ACTIVATION SYSTEM: S-9 mix prepared from adult male rat liver induced by Aroclor 1254
SOLVENT: water
CONTROLS: negative (without TS) and positive controls (EMS)
MEDIA:
- Culture medium: Fischer's mouse leukemia medium supplemented with pluronic solution, L-glutamine, sodium pyruvate, antibiotics, and horse serum
- Cloning medium: as culture medium minus pluronic solution, with addition of agar to achieve semisolid state
- Selection medium: cloning medium containing 100 µg/ml of BrdU or 3 µg/ml of TFT
PERFORMANCE OF TEST:
- Exposure to TS: 10 to 15 concentrations; exposure 4 hours
- Expression of TK-/- phenotype: washing and placing of cells in growth medium for 2-3 days; selection of doses for mutant analysis
- Mutant analysis: selected doses seeded in soft agar plates with selection medium; mutant colonies counting after ca. 10 days incubation

MINIMUM CRITERION (to demonstrate mutagenesis): mutant frequency that is at least 150% of the concurrent background frequency (average of solvent and negative controls) plus 10 x 10E-6.

EVALUATION CRITERIA FOR REACHING CONCLUSION "MUTAGENIC":
- Dose-related or toxicity-related increase in mutant frequency
- If an increase in mutant frequency is followed by smaller or no increases at higher concentrations, values must not be below the minimum criterion

- no statistics performed
- Increase of about two times the minimum criterion or greater for a single dose near the highest testable toxicity

Reliability: (1) valid without restriction
Parameters described closely comparable to OECD Guideline 476; study performed according to GLP

Flag: Critical study for SIDS endpoint

12-JAN-2004 (69)

Type: HGPRT assay
System of testing: Chinese hamster ovary (CHO) cells-KTL3
Concentration: 11.8; 23.7; 35.5; 47.3 µM/ml (2; 4; 6; 8 µg/ml)
Cytotoxic Concentration: 8 µg/ml
Metabolic activation: without
Result: positive

Method: other: according to Jansson K. and Hyttinen J. M. T.: Mut. Res., 322, 129-132 (1994)
Year: 1995
GLP: no
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Result: MUTANT FREQUENCIES (number of mutants per 10E-6 clonable cells; average of 2 trials; * P<0.05):
3; 12; 19*; 17*; 40* at 0; 2; 4; 6(only trial #2); and 8 µg/ml, resp. (positive control: 321*)

CLONING EFFICIENCY (in %; values of trial #1 and #2):
- on day after treatment: 78/75; 63/79; 74/61; -/26; 3/2 at 0; 2; 4; 6(only trial #2); and 8 µg/ml, resp. (positive control: 60/73)
- at time of mutant selection: 70/76; 72/82; 81/76; -/69; 68/83 at 0; 2; 4; 6(only trial #2); and 8 µg/ml, resp. (positive control: 86/83)

EVALUATION OF RESULTS
Positive because of statistically significant linear relationship between concentration and mutant frequency

Test condition: METABOLIC ACTIVATION SYSTEM: presumably without, but not explicitely stated
SOLVENT: water
CONTROLS: negative (vehicle control without TS) and positive control (500 µg/ml EMS)
MEDIA:
- Culture medium: MEM-alpha medium without ribonucleosides or deoxyribonucleosides, supplemented with 10% fetal calf serum and antibiotics
- Cloning medium: culture medium without serum and antibiotics
NUMBER OF REPLICATES: 2 trials

PERFORMANCE OF TEST:
- Exposure to TS 3 hours at 37°C
- After washing of cell monolayers and additional 18 hour incubation in complete medium, dissociation of cells with 0.05% trypsin and 0.02% EDTA
- Mutant analysis: 1x10E6 cells plated per 175cm² flask (2 flasks per dose) in 35 ml of medium, subcultured 3 days later , and incubated for a total period of 6 days. 2x10E5 cells then plated per 80 cm² flask (8 flasks per dose) in 16

ml of medium containing 30 µM 6-thioguanine. Mutant colonies fixed and stained 8 days later.
- Cloning efficiency: determined on day after treatment and at time of mutant selection by plating 200 cells per 25 cm² flask (4 flasks per dose)

STATISTICAL ANALYSIS: Fisher's protected least-significant difference test (ANOVA followed, if significant, by LSD); level of significance: P <0.05

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; documentation of experimental details limited to the above.

Flag: Critical study for SIDS endpoint

03-JUL-2003 (70)

Type: Micronucleus test in vitro
System of testing: V79 Chinese Hamster Lung Cells
Concentration: 0.313-17.5 µg/ml (see freetext Test conditions for further details)
Cytotoxic Concentration: >= 25 µg/ml (with/without MA, pretests)
Metabolic activation: with and without
Result: positive

Method: other: Draft OECD guideline 1998 (see freetext)
Year: 2001
GLP: yes
Test substance: other TS: Mucochloric acid techn. pure (2 x recrystallized) obtained from BASF AG, purity 99.3%

Method: Test according to a proposal for a Draft OECD guideline for the in vitro micronucleus test (1998).
Literature:
Kallweit et al., 1999, Mut Res 439, 183-199
Seelbach et al., 1993, Mut Res 303, 163-169
Seelbach et al., 1993, Toxicol. in Vitro 7, 185-193

Result: TREATMENT CONDITIONS:
Osmolality and pH values not influenced by the treatment.

GENOTOXIC EFFECTS (Mixed population method):
- Without metabolic activation dose-dependent increase in number of micronucleated cells from about 4 µg/ml onward:
Mean micronucleus frequency was:
1.3; 0.75; 0.75; 0.7; 1.55% at 0; 0.313; 0.625; 1.25; 2.5 and 5 µg/ml, resp. (1st trial)
0.55; 0.8; 1.05; 2.45; 4.2; 3.9% at 0; 2; 3; 4; 5 and 6 µg/ml, resp. (2nd trial)
- With metabolic activation number of micronucleated cells close to the range of concurrent negative control and within range of historical control data (0.9+/-0.3%). Mean micronucleus frequency was:
0.7; 1.25; 0.8; 1.05; 0.95; 0.65% at 0; 0.625; 1.25; 2.5; 5 and 10 µg/ml, resp.

CYTOTOXIC CONCENTRATION:
No suppression of the mitotic activity at any dose level (determination of mitotic index); no indication of

cytotoxicity based on determination of the proliferation index and cell counts.
Cell attachment and quality of cells were influenced without MA from about 2.5 µg/ml up to 7 µg/ml and with MA at 10 µg/ml.

ANEUGENIC EFFECTS (Mitotic shake off method):
No increase in the number of cells containing micronuclei at any dose level.

CONTROLS:
Spontaneous micronuclei in negative controls within the normal range; valid positive controls.

EVALUATION:
Under the experimental conditions of this study the TS is a micronucleus-inducing (clastogenic) agent.

Test condition: METABOLIC ACTIVATION (MA) SYSTEM:
S-9 mix with liver homogenate from male Sprague-Dawley rats treated with 500 mg/kg Aroclor1254 5 d prior to sacrifice

CONTROLS:
concurrent vehicle controls (only culture medium; no other vehicle used); concurrent positive controls ethyl methanesulfonate (without MA) and cyclophosphamide (with MA); colcemid (for detection of aneugens without MA in mitotic shake off method)

MEDIA:
- Culture medium: MEM medium with 10% fetal calf serum + 1% penicillin/streptomycin and 1% amphotericine
- Treatment medium: same medium without fetal calf serum

PRETESTS FOR DOSE SELECTION:
Range-finding test in the range of 1.0 - 1700 µg/ml both without (continuous treatment of 24 h) and with MA (pulse treatment of 4 h). Based on cell count, cell attachment and quality of cells (cytotoxicity >=25 µg/ml) the following top doses were selected for the tests along the mixed population method: 7.5 µg/ml without MA, 10 µg/ml with MA.

PERFORMANCE OF TEST:
No specific vehicle used except for the aqueous culture medium.
In all exposure groups duplicate cultures used; 1000 cells per culture analysed.

Experiments using mixed population method:
a) 24 h exposure, 24 h harvest time, without S9-mix;
concentrations 1st trial: 0; 0.313; 0.625; 1.25; 2.5; 5 µg/ml; 2nd trial (repeat): 0; 2; 3; 4; 5; 6 µg/ml
b) 4 h exposure, 24 h harvest time, with S9-mix;
concentrations 0; 0.625; 1.25; 2.5; 5; 10 µg/ml

Experiments using the mitotic shake off method (3 h mitotic shake off; 3 h exposure; 6 h harvest time; only without S9-mix):
concentrations 1st trial: 0; 3; 4; 5; 6; 7 µg/ml
concentrations 2nd trial (repeat): 0; 7.5; 10; 12.5;15; 17.5 µg/ml

STATISTICS:
No statistical analysis due to the clear positive findings.

EVALUATION CRITERIA:

Test substance is considered positive if:
- Dose-related and reproducible significant increase in number of cells containing micronuclei;
- Proportion of micronucleus-containing cells exceeded both the concurrent negative control range and negative historical control range.
Test substance is generally considered nongenotoxic if:
- No significant increase in number of micronucleus-containing cells at any dose above concurrent negative control frequencies and within the historical control data.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
12-JAN-2004 (71)

Type: Ames test
System of testing: Salmonella typhimurium TA 100
Concentration: 0.625 ug - 20 ug/plate
Cytotoxic Concentration: without activation: >= 10 µg/plate; with activation no cytotoxicity
Metabolic activation: with and without
Result: ambiguous

Method: other: according to Ames B.N. et al.: Proc.Nat.Acad.Sci. USA, 70, 2281-2285 (1973) and Ames B.N. et al.: Mut.Res., 31, 347-364 (1975)
Year: 1985
GLP: no
Test substance: other TS: Mucochloric acid, technical grade, purity 97.6%

Remark: Result: positive in trials without metabolic activation from 2.5 ug/plate
Result: MCA:
without activation:
- First experiment: 0.9; 1.3; 2.5 and 3.7 fold higher than negative control at 0.625; 1.25; 2.5 and 5 µg/plate
Positve control: MNNG: 16.4 fold higher than negative control;

- Second experiment: 1.0; 1.0; 1.5; 2.2 and 1.6 fold higher than negative control at 0.625; 1.25; 2.5; 5 and 10 µg/plate
Positive control: MNNG: 14.4 fold higher than negative control
with activation:
- first experiment: contaminated
- second experiment: 1.0; 1.0; 1.1; 1.1; 0.8 and 0.8 fold higher than negative control at 0.625; 1.25; 2.5; 5; 10 and 20 µg/plate
Positive control: 2-AA 23.7 fold higher than negative control
Test condition: SYSTEM OF TESTING
- Type: plate incorporation assay
- Metabolic activation system: S-9 mix prepared from livers of male Sprague-Dawley rats induced by Aroclor 1254
- Number of plates: 3 test plates per dose or per control
SOLVENT: DMSO
CONTROLS: negative (solvent and sterility) controls;
positive controls: 2-aminoanthracene (with S-9 mix), MNNG (without S-9 mix)

- 2 experiments with and without activation
- Incubation for 48 hours at 37°C
EVALUATION CRITERIA:
a) doubling of the spontaneous mutation rate (control)
b) dose-response relationship
c) reproducability of the results
- no information on statistical methods available

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; limitations study not performed according to GLP

12-JAN-2004 (72)

Type: Ames test
System of testing: Salmonella typhimurium TA 100
Concentration: 0.625 ug- 20 ug/plate
Cytotoxic Concentration: without activation: >= 10 µg/plate; with activation no cytotoxicity
Metabolic activation: with and without
Result: positive

Method: other: according to Ames B.N. et al.: Proc.Nat.Acad.Sci. USA, 70, 2281-2285 (1973) and Ames B.N. et al.: Mut.Res., 31, 347-364 (1975)
Year: 1985
GLP: no
Test substance: other TS: Mucochloric acid, technical grade; purity: 97.5%

Remark: Result: positive in trials without metabolic activation from 1.25 ug/plate
Result: MCA:
without activation:
- First experiment: 1.2; 1.7; 2.4; 3.7 and fold higher than negative control at 0.625; 1.25; 2.5; 5 and 10 µg/plate
Positve control: MNNG: 16.4 fold higher than negative control;

- Second experiment: 1.2; 1.7; 1.5; 2.1; 2.5 and 1.6 fold higher than negative control at 0.625; 1.25; 2.5; 5; 10 and 20 µg/plate
Positive control: MNNG: 14.4 fold higher than negative control
with activation:
- First experiment: contaminated
- Second experiment: 0.8; 1.0; 1.2; 1.2; 1.2 and 1.3 fold higher than negative control at 0.625; 1.25; 2.5; 5; 10 and 20 µg/plate
Positive control: 2-AA 23.7 fold higher than negative control
Test condition: SYSTEM OF TESTING
- Type: plate incorporation assay
- Metabolic activation system: S-9 mix prepared from livers of male Sprague-Dawley rats induced by Aroclor 1254
- Number of plates: 3 test plates per dose or per control
SOLVENT: DMSO
CONTROLS: negative (solvent and sterility) controls;
positive controls: 2-aminoanthracene (with S-9 mix), MNNG (without S-9 mix)
- Incubation for 48 hours at 37°C
- 2 experiments with and without activation
EVALUATION CRITERIA:
a) doubling of the spontaneous mutation rate (control)
b) dose-response relationship
c) reproducability of the results

- no information on statistical methods available

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; study was performed prior to GLP implementation

12-JAN-2004 (72)

Type: Ames test
System of testing: Salmonella typhimurium TA 100
Concentration: no information given
Cytotoxic Concentration: no information given
Metabolic activation: with and without
Result: positive

Method: other: Ames BN et al. (1975) Mutat Res 31: 347-363, Maron DM, Ames BN (1983) Mutat Res 113: 173-215
Year: 1988
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Result: Net TA-100 revertants/nmol
MCA:
- without metabolic activation 60
- with metabolic activation 5.0

Positive without metabolic activation between 5 and 30 nmol/plate (= 0.8845 - 5.07 µg/plate). Mutagenicity reduced after addition of metabolic system.

Test condition: METABOLIC ACTIVATION (MA) SYSTEM:
S-9 mix with liver homogenate from rats treated with Aroclor1254

CONTROLS:
no information given

Minimum 3 plates per dose level
all tests performed in duplicate

EVALUATION:
- values for revertants/nmol obtained from linear portion of the dose-response curves and reported as net (i.e. induced) revertants after correction for the spontaneous TA 100 mutants which averaged 159.
- no information on statistical methods given
- Acceptance criteria: results only reported if the dose-response curve was reproducible and number of spontaneous TA 100 mutants in the range of 100-200 (historical norm of laboratory)

Details on test conditions limited to the above

Reliability: (2) valid with restrictions
Scientifically acceptable study despite of limited experimental details in documentation

Flag: Critical study for SIDS endpoint

29-APR-2004 (73) (74)

Type: Ames test
System of testing: Salmonella typhimurium TA 100
Concentration: 0.1; 1; 10; 100; 1000 ug/plate

Cytotoxic Concentration: no information given
Metabolic activation: no data
Result: positive

Method: other: no information given
Year: 1980
GLP: no
Test substance: no data

Result: no further details on study outcome given
Test condition: no further details on test method given
Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test procedure and results)
28-JAN-2004 (75)

Type: Ames test
System of testing: Salmonella typhimurium TA 1535 TA 1537 TA 1538 TA 98 TA 100
Concentration: 0.5; 2.0; 7.8; 31.3; 125 ug/plate
Cytotoxic Concentration: with metabolic activation: 125 µg/plate in TA 1535; without metabolic activation: >=31.3 µg/plate in all tester strains
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 1983
GLP: yes
Test substance: no data

Result: without metabolic activation:
- TA 1535: 13; 10; 12; 10; 0; 0 revertants at 0; 0.5; 2; 7.8; 31.3 and 125 µg/plate; positive control: 1820 revertants
- TA 1537: 15; 12; 14; 16; 9; 0 revertants at 0; 0.5; 2; 7.8; 31.3 and 125 µg/plate; positive control: 151 revertants
- TA 1538: 13; 9; 9; 11; 8; 0 revertants at 0; 0.5; 2; 7.8; 31.3 and 125 µg/plate; positive control: 170 revertants
- TA 98: 22; 20; 21; 39; 0; 0 revertants at 0; 0.5; 2; 7.8; 31.3 and 125 µg/plate; positive control: 128 revertants
- TA 100: 90; 89; 95; 116; 0; 0 revertants at 0; 0.5; 2; 7.8; 31.3 and 125 µg/plate; positive control 2076 revertants

with metabolic activation:
- TA 1535: 10; 8; 4; 6; 7; 0 revertants at 0; 0.5; 2; 7.8; 31.3 and 125 µg/plate; positive control: 569 revertants
- TA 1537: 23; 17; 14; 15; 17; 11 revertants at 0; 0.5; 2; 7.8; 31.3 and 125 µg/plate; positive control: 153 revertants
- TA 1538: 29; 25; 29; 20; 29; 21 revertants at 0; 0.5; 2; 7.8; 31.3 and 125 µg/plate; positive control: 1331 revertants
- TA 98: 60; 52; 56; 50; 48; 44 revertants at 0; 0.5; 2; 7.8; 31.3 and 125 µg/plate; positive control: 2113 revertants
- TA 100: 104; 101; 102; 100; 95; 88 revertants at 0; 0.5; 2; 7.8; 31.3 and 125 µg/plate; positive control 1906 revertants

Test condition: Testing of every concentration in triplicates

Negative controls: untreated and vehicle control (DMSO)

Vehicle: water

Positive controls:

without metabolic activation:
- TA 1535 and TA 100: MNNG (1.6 µg)
- TA 1537: 9-aminoacridine (50 µg)
- TA 1538: 4-nitroquinoline-N-oxide (0.25 µg)
- TA 98: daunomycine (5 µg)
with metabolic activation:
- all strains: 2-anthramine (12.5 µg)

Evaluation criteria:
- doubling of the spontaneous reversion rate
- dose-effect relationship

documentation of the test method limited to above; no information on test substance purity given

Reliability: (3) invalid
Significant methodological deficiences: no data on test substance purity reported; no information on solubility; precipitation

12-JAN-2004 (76)

Type: Ames test
System of testing: Salmonella typhimurium TA 100
Concentration: see freetext
Cytotoxic Concentration: pH 7: without metabolic activation: 100 µg/plate (592 nmole/plate); with activation: 300 µg/plate (1667 nmole/plate)
pH 6: without metabolic activation: 10 µg/plate (60 nmole/plate)
Metabolic activation: with and without
Result: positive

Method: other: according to Maron and Ames: Mut. Res., 113, 173-215 (1983)
Year: 1986
GLP: no data
Test substance: other TS: Mucochloric acid, purity 99%

Remark: The mutagenicity was tested at different pH values.
Number of revertants per nmol: 5.53; 1.17; 0.54 at pH 6, 7 and 8, resp.
Result: - Addition of S9-mix reduced mutagenicity at low dose levels (up to approx. 30 µg/plate) but extended the range at which activity was detectable due to reduction of cytotoxicity (experiments performed at pH 7.0)

PH DEPENDENT MUTAGENICITY:
pH 6: 146 and 312 revertants per plate at 0 and 5 µg/plate; cytotoxicity at 10; 15 and 20 µg/plate; TA 100 net revertants per nmole 5.53
pH 7: 156; 206; 247; 277 and 294 revertants per plate at 0; 5; 10; 15 and 20 µg/plate; TA 100 net revertants per nmole 1.17
pH 8: 108; 129; 137; 156 and 175 revertants per plate at 0; 5; 10; 15 and 20 µg/plate; TA 100 net revertants per nmole 0.54
Test condition: - Testing of 50 and 100 µl doses of HPLC fractions
- additional tests with modification of pH by adjusting of pH of the base agar to either 6 or 8 according to method by Popkin DJ and Prival MJ (1985). Mutat. Res. 142: 109-113.
- metabolic activation with S9-mix

Experiments at pH 7:
- Testing in dublicate plates per dose

- dose levels: without activation: 1-100 µg/plate (6-592 nmol/plate); with activation: 1- 300 µg/plate (6 - 1776 nmol/plate

Experiments with different pH 6, 7, 8:
- Testing in triplicate plates per dose
- calculation of TA 100 net revertants per nmole based on the 30 nmole values at pH 6 and from slope of dose-response curves at pH 7 and pH 8
- dose level without activation: 30; 60; 90 and 120 nmol/plate (5; 10; 15 and 20 µg/plate)

- no further methodological details given

Reliability: (2) valid with restrictions
Scientifically acceptable study despite of limited experimental details in documentation

12-JAN-2004 (77)

Type: Ames test
System of testing: Salmonella typhimurium TA100
Concentration: range: 0-40 µg/plate for details see freetext
Cytotoxic Concentration: 40 µg/plate
Metabolic activation: without
Result: positive

Method: other: according to Maron and Ames: Mut. Res., 113, 173-215 (1983)
Year: 1990
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Result: NET REVERTANTS PER µMOL:
- Test A: 4.021; Test B: 9.276; Test C: 7.243

Test condition: DOSE LEVELS:
- MCA: Test A: 0; 5; 10; 20; 40 µg/plate; Test B: 0; 2; 4; 8; 10 µg/plate; Test C: 0; 2; 4; 6; 8; 10 µg/plate
- Three plates per dose level

SOLVENT: DMSO

MUTAGENICITY OF THE COMPOUNDS:
- expressed as revertants/µg/plate; calculated by least-squares regression analysis of the linear part of the dose-response curve where spontaneous TA 100 mutants from DMSO controls were taken as the zero-dose point

CONTROLS:
- Positive Control: MMS
- Solvent Control: DMSO

Details on test conditions limited to the above

Reliability: (2) valid with restrictions
Scientifically acceptable study despite of limited experimental details in documentation

12-JAN-2004 (78)

Type: Ames test

System of testing: Salmonella typhimurium TA 100
Concentration: up to 2000 ng/plate
Cytotoxic Concentration: 2000 ng/plate highest non cytotoxic dose
Metabolic activation: no data
Result: positive

Method: other: according to Maron and Ames: Mut. Res., 113, 173-215 (1983)
Year: 1995
GLP: no
Test substance: other TS: Mucochloric acid, purity 98%

Remark: Comparative investigation on MX and MCA
Result: MCA:
- highest non toxic dose observed 2,000 ng/plate
- Number of reversion events at the his G46 codon at 2,000 ng/plate: total mutants 104; GC->AT transition: TCC: 15, CTC: 54; GC->TA transversion: ACC: 3, CAC 29; GC -> CG transversion: GCC: 1; Extragenic suppressors: CCC: 2

MX:
- highest non toxic dose observed 400 ng/plate
- Number of reversion events at the his G46 codon at 400 ng/plate: total mutants 114; GC->AT transition: TCC: 4, CTC: 8; GC->TA transversion: ACC: 26, CAC 73; GC -> CG transversion: GCC: 2; Extragenic suppressors: CCC: 1

SOLVENT CONTROL (DMSO):
- Number of reversion events at the his G46: total mutants 137; GC->AT transition: TCC: 15, CTC: 28; GC->TA transversion: ACC: 18, CAC 75; GC -> CG transversion: GCC: 0; Extragenic suppressors: CCC: 1

MX predominantely induced GC->TA transversions with a 3:1 preference for the second position of the his G46 (CCC) codon. The mutational spectrum of MCA was significantly different from that induced by MX. MCA induced primarily GC->AT transitions with a 4:1 preference for the second position of the his G46 codon

Test condition: AMES TEST:
- Test Strain: S. thyphimurium TA 100 (hisG46, rfa, deltauvrB, pKM101)
- Standard plate test performed in triplicates
- Solvent control: DMSO 100 µl/plate

DNA COLONY HYBRIDIZATION.
- according to method of Cebula and Koch (1990) Mutat Res 229: 79-87 without Psoralen crosslinking step (Maragos CM, Andrews AW, Keffer LK, Elespuru RK (1993) Mutat Res 298: 187-195.
- Probes: TCC, CTC, ACC, CAC, GCC and CCC 5'-(32P) end-labelled oligonucleotides (15-mer)
- Filters: Whatman 541 colony lift filters
- Hybridization conditions: 47 °C for 3 hours exept GCC probe hybridized at 50 °C for 3 hours
- Washing 3 x SCC (0.15 M NaCl and 0.015 M sodium citrate pH 7.0) at 47 °C for 12 min, TCC probe 25 min, GCC probe 1 x SCC at 50 °C for 17 min
- Drying of filters
- Autoradiographing using intensifier screens

STATISTICS:

- Chi² analysis using SPSS/PC+ V5.0 program package

Details on test condition limited to the above
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Documentation limited to the above.
Flag: Critical study for SIDS endpoint
28-JAN-2004 (79)

Type: Ames test
System of testing: Salmonella typhimurium TA 100
Concentration: no information given
Cytotoxic Concentration: no information given
Metabolic activation: without
Result: positive

Method: other: according to Ames B.N. et al.: Mut. Res., 31, 347-364 (1975)
Year: 1975
GLP: no
Test substance: no data

Result: MCA: 3.6 revertants / nmol
Test condition: no methodological details given
Reliability: (4) not assignable
Documentation not sufficient for assessment (limited information on test procedure and results)
12-JAN-2004 (80)

Type: Ames test
System of testing: Salmonella typhimurium TA 100, TA 1535
Concentration: 2 - 17 nmol/plate (0.3 - 2.7 µg/plate)
Cytotoxic Concentration: no information given
Metabolic activation: no data
Result: positive

Method: other: according to Maron D. M. and Ames B. N.: Mut. Res., 113, 173-215 (1983)
Year: 1995
GLP: no
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Remark: - Suspectability to MX mutagenicity in T 100 strain highly increased in comparison to parent strain 1535
- no such difference in suspectability to MCA mutagenicity
Result: TA 100 strain:
- dose dependent increase in the range from approx. 100 to approx. 300 revertants per plate
- Potency (= slope of the least square lines): MCA 23; MX 3,900 revertants/nmol

TA 1535 strain:
- dose dependent increase in the range from approx. -20 to approx. 170 revertants per plate
- Potency (= slope of the least square lines): MCA 9; MX 19 revertants/nmol

Suspectability to MX mutagenicity in T100 strain highly

increased in comparison to parent strain TA 1535
no such difference in suspectability to MCA mutagenicity

Test condition: STANDARD PLATE INCORPORATION TEST:
- performed in triplicate
- test substances MCA and MX were added to the top agar in 100 µl DMSO
- no further details on test method given

Reliability: (2) valid with restrictions
Scientifically acceptable study despite of limited experimental details in documentation

28-JAN-2004 (70)

Type: other: DNA Damage shown as single and double strand brakes
System of testing: PHIX174 DNA
Result: positive

Method: other: chemical reactivity of MCA with PHIX174 DNA
Year: 1997
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Result: - MCA converted closed supercoiled plasmid PHIX174 DNA to its relaxed form (indicator for single strand brakes) and linear forms (indicator for double strand brakes). Further destruction of the plasmid DNA towards smaler DNA fractions was also observed the longer the incubation period was
- ionic strength had no effect on the reaction
- the kinetic of this reaction indicated sequential single-strand cleavage as the mode of action oppose to double strand cleavage for the production of L-PHIX174

MX, RMCA as well as MMS and NaN3 converted the plasmid DNA only to the relaxed but not to the linear form.
MCA-IPE had practically no effect on PHIX174
Increasing concentratuions of GSH deminished the cleavage of supercoiled PHIX174 by MCA, but increased the cleavage by MX.

The action of MCA was unique,
among several stronger and weaker mutagens investigated in this assay.

Test condition: BUFFER:
- TNE-Buffer: aqueous solution of Tris-HCl (10 mM, pH 8.0), NaCl (100 mM) and EDTA (1mM, pH 8.0)
- TE-Buffer: aqueous solution of Tris-HCl (10 mM, pH 8.0) and EDTA (1mM, pH 8.0)

GENERAL PROCEDURE:
- Stock solutions of mutagens (13.6 mM) are prepared immediately before use in TNE-buffer followed by subsequent dilution in TNE-buffer
- pH adjustment to pH 8.0
- Stodck solutions of PHIX174 plasmid DNA (91 ng/µl in TE pH 7.8) were prepared and stored at -20 °C
- Analysis supercoiled (SC), relaxed (R) and linear (L) DNA by horizontal agarose (0.7%) gel electrophoresis
- Visualization of bands by ethidium bromid staining; concentration in gel and running buffer 0.5 µg/ml
- Gels viewed under UV and polaroid photographs taken using positive and negative type 55 film; film negatives fixed with

18% Na2SO3 solution
- Quantification of relative concentrations of DNA bands with Hoefer densitometer linked to an IBM/PS2 computer for storage and manipulation of data
- Location of L-PHIX174 DNA by comparison with Hind 111 DNA molecular weight marker
- Preparation of L-PHIX174 DNA by cutting SC-PHIX174 DNA with Pst 1 restriction enzyme
- Preparation of R-PHIX174 DNA by 10 min UV irradiation of SC-PHIX174 DNA

TIME EFFECT OF MCA ON PHIX174:
- 6.4 µl TE solution of PHIX174 was combined with 24 µl TNE solution: 74.3 ng/µl PHIX174; 4.1 mM (0.69 mg/ml)
- Control solution: 10 µl TNE solution containing 74.3 ng PHIX174
- Incubation at 37 °C
- Collection of each of 5 10 µl aliquots of the MCA solution after 3.5; 8.25; 17.5; 21.5 and 24 hours; each aliquot was frozen at -78 °C immediately after beeing withdrawn
- Incubation of control solution for 24 hours and subsequent freezing
- Thawing of samples for analysis
- Treatment with 3.5 µl of run/stop (R/S) solution
- 10 µl of each of the MCA-PHIX174 reaction samples, the control solution, the UV-relaxed PHIX174 solution and the Hind 111 marker were loaded on the wells of a agarose gel
- Electrophoresis at 25 V for 0.5 h and then at 75 V until the dye had transversed 2/3 the gel length
- Photographing of the gels
- Quantification of relative amounts of SC, R and L PHIX174 DNA by densitometry

CONCENTRATION EFFECT OF MCA ON PHIX174:
a)
- 5 vials containing 3.2 µl TE solution of PHIX174 (291 ng) were added by TNE solution of MCA to obtain final concentrations of 0.68; 1.36; 2.73; 4.09 and 5.46 mM MCA (corresponding to 0.11; 0.23; 0.46; 0.69 and 0.92 mg/ml); the volume wasmade up to 39.2 µl
- a sixth vial contained the control solution consisting of 3.2 µl of PHIX174 (291 ng) in 39.2 µl of TNE buffer
- Incubation at 37 °C for 24 hours
- Mixing of 10 µl of a vial together with 3 µl of R/S solution in a second vial
- A 10 µl aliquot (57.1 ng) of each vial was loaded into the wells of an agarose plate together with the control solution the Hind 111 marker and a Zero MCA control (MCA-solution that was not incubated)
- Electrophoresis at 25 V for 0.5 h and then at 75 V until the dye had transversed 2/3 the gel length
- Photographing of the gels
- Quantification of relative amounts of SC, R and L PHIX174 DNA by densitometry
b)
- 15 vials containing 1.6 µl TE solution of PHIX174 (291 ng) were added by TNE solution of MCA to obtain final concentrations of 0; 0.36; 0.72; 1.08; 1.44; 1.8; 2.16; 2.52; 2.88; 3.23; 3.59; 3.95; 4.31; 5.39 and 5.75 mM MCA (corresponding to 0.06, 0.12; 0.18; 0.24; 0.30; 0.36; 0.43; 0.49; 0.55; 0.61; 0.67; 0.73; 0.91; 0.97 mg/ml); the volume

was made up to 19.6 µl
- a six vial contained the control solution consisting of 3.2 µl of PHIX174 (291 ng) in 39.2 µl of TNE buffer
- Incubation at 37 °C for 24 hours
- Mixing of 10 µl of a vial together with 3 µl of R/S solution in a second vial
- A 10 µl aliquot (57.1 ng) of each vial was loaded into the wells of an agarose plate together with the control solution the Hind 111 marker and a Zero MCA control (MCA-solution that was not incubated)
- Electrophoresis at 25 V for 0.5 h and then at 75 V until the dye had transversed 2/3 the gel length
- Photographing of the gels
- Quantification of relative amounts of SC, R and L PHIX174 DNA by densitometry

MCA INTERACTION WITH L-PHIX174:
- Preparation of L-PHIX174: 3 µg of SC-PHIX174 was cut with restriction enzyme Pst1 to obtain L-PHIX174
- Purification of L-PHIX174: Precipitation with ethanol and redissolvance in TE buffer
- Preparation of reaction mixtures:
a) 600 ng (2 µl) of L-PHIX174 and 18 µl TNE-buffer
b) 600 ng (2 µl) of L-PHIX174, 8 µl of MCA (6.69 mM; 1.13 mg/ml) in TNE-buffer and 10 µl TNE-buffer
c) 900 ng (3 µl) of L-PHIX174, 8 µl of MCA (6.69 mM; 1.13 mg/ml) in TNE-buffer and 9 µl TNE-buffer
- Incubation at 37 °C
- Sample collection after 12 and 24 h (10 µl); 12 h samples were frozen (-78 °C) until after the 24 h samples were taken
- Addition of 3 µl of R/S solution to 24 h and thawed 12 h samples of reaction and control solutions
- Gel loading, electrophoresis and densitometry as previously described

EFFECTS OF MCA, METHYL METHANESULFONATE (MMS) AND NAN3 ON PHIX174:
- Preparation of stock solutions: 14.54 mM (2.46 mg/ml) MCA, 21.71 mM MMS, 13.37 mM NaN3 in TNE buffer and control solution without mutagen
- Separate incubation of MCA (4.07 mM; 0.69 mg/ml), MMS (3.74 mM),NaN3 (4.07 mM) and control solution with 582 ng (6.4 µl) PHIX174 in a total volume TNE-buffer of 78.4 µl at 37 °C
- Sample collection after 12 and 20 h and transfer into new vials (10 µl); 12 h samples were frozen (-78 °C) until after the 20 h samples were taken
- Addition of 3 µl of R/S solution to 20 h and thawed 12 h samples of reaction and control solutions
- Gel loading, electrophoresis and densitometry as previously described

EFFECTS OF MCA, REDUCED MCA (RMCA) AND MCA ISOPROPYLETHER (MCA-IPE) ON PHIX174:
- Preparation of three vials with 291 ng PHIX174 in a total volume of 39.2 µl TNE-buffer; one containing 4.19 mM (0.71 mg/ml) MCA, the next containing 4.18 mM RMCA and the last 4.17 mM MCA-IPE
- Control solutions: PHIX174 in TNE buffer without mutagen; Solvent water:DMSO (12:1)
- Incubatiton at 37 °C
- Sample collection after 12 and 22 h and transfer into new

vials (11µl); 12 h samples were frozen (-78 °C) until after the 22 h samples were taken
- Addition of 3 µl of R/S solution to 22 h and thawed 12 h samples of reaction and control solutions
- Gel loading, electrophoresis and densitometry as previously described

GLUTATHION EFFECT ON THE INTERACTION OF MCA WITH PHIX174:
- Preparation of five vials with 291 ng PHIX174 and 1.41 mM (0.24 mg/ml) MCA in a total volume of 39.2 µl TNE-buffer; GSH concentrations in the five vials were 0.17; 0.34; 0.68; 1.02; 1.42 mM
- Preparation of three control solutions with 291 ng PHIX174 in a total volume of 39.2 µl: the first contained PHIX174 only; the second contained additionally MCA (1.41 mM; 0.24 mg/ml); the third contained PHIX174 and GSH (1.42 mM)
- Incubatiton at 37 °C for 24 h
- Sample collection after 24 h (10 µl)
- Addition of 3 µl of R/S solution to samples of reaction and control solutions
- Gel loading, electrophoresis and densitometry as previously described

Conclusion: Formation of linear DNA was attributed to two sequential single strand brake steps

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments

Flag: Critical study for SIDS endpoint

08-JUL-2003 (81)

Type: other: Single-Cell Gel / Comet Assay
System of testing: Chinese hamster ovary (CHO) cells
Concentration: 0; 3.7; 7.4; 14.8; 29.6; 59.2; 118.4 µM corresponding to 0.63; 1.25; 2.50; 5.00; 10.00; 20.00 µg/ml for details see free text on test conditions
Cytotoxic Concentration: survival (vs. control) in all concentrations > 75%
Metabolic activation: without
Result: positive

Method: other: Singh et al. (1988) with some modifications
Year: 2001
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Remark: Comparative study of chlorohydroxyfuranones including MCA and MX

Result: MCA:
- tested only in non respictively minor cytotoxic concentrations 0.63; 1.25; 2.50; 5.00; 10.00; 20.00 µg/ml.
(* p<0.05, ** p<0.01, *** p<0.001 vs. negative control two-tailed t-test)

Tail %DNA:
13.52; 15.51; 10,28; 12.06; 16.82*; 24.29***; 32.10***
positive control 45.65*** at 150 µg/ml

Tail extent moment:
6.24; 7.12; 4.56; 5.79; 7.54; 11.58***; 17.12***
positive control 25.73*** at 150 µg/ml

Olive tail moment:
2.19; 2.66; 1.80; 2.27; 2.98**; 4.66***; 7.26***
positive control 9.90*** at 150 µg/ml

Tail length:

41.57; 40,98; 41,42; 39.13; 40.83; 44.09; 51.61***
positive control 55.14*** at 150 µg/ml

MX:
- tested only in non respictively minor cytotoxic concentrations 0; 0.50; 1.00; 2.00; 4.00; 8.00; 16.00; 32.00 µg/ml
(* p<0.05, ** p<0.01, *** p<0.001 vs. negative control two-tailed t-test)

Tail %DNA:
9.18; 10.80; 9.84; 9.89; 10.68; 16.54***; 34.58***; 39.67***
positive control 40.49*** at 150 µg/ml

Tail extent moment:
3.71; 4.66; 3.69; 4.01; 4.37; 6.82***; 17.91***; 22.28***
positive control 21,38*** at 150 µg/ml

Olive tail moment:
1.46; 1.60; 1.46; 1.53; 1.62; 2.73***; 7.73***; 9.55***
positive control 7.89*** at 150 µg/ml

Tail length:
37.96; 39.67; 34.98; 36.31; 36.91; 38.29; 49.70***; 54.84***
positive control 51.34*** at 150 µg/ml

Test condition: TEST SUBSTANCES:
MCA
MX:
- Purity (NMR): >= 98%
- Concentrations: 0; 2.3; 4.6; 9.2; 18.4; 36.8; 73.6; 147.2 µM corresponding to 0; 0.50; 1.00; 2.00; 4.00; 8.00; 16.00; 32.00 µg/ml
POSITIVE CONTROL:
- Methyl methanesulfonate (MMS)
- Concentration: 1362 µM corresponding to 150 µg/ml

METABOLIC ACTIVATION SYSTEM: presumably without, but not explicitely stated
SOLVENT: PBS
CONTROLS: negative (solvent control: PBS) and positive control (Methyl methanosulfonate (MMS))
MEDIA:
MEM-alpha medium, supplemented with 10% fetal calf serum, penicillin (100 IU/ml, and streptomycin 100 µg/ml)
SELECTION OF TEST SUBSTANCE CONCENTRATIONS: to cause only a minor or no decrease in survival (survival > 75%) or in the number of harvested cells compared to concurrent control cultures.

NUMBER OF REPLICATES: 1 (dublicate flasks per dose group)

PERFORMANCE OF TEST:

- Preincubation of 0.8 x 10E6 CHO cells in 25 cm² flasks in

dublicate, 24 hours before treatment.
- Exposure to TS or controls (100 µl) for 1 hour in PBS without CA++ and Mg++
- Harvesting of cells: By flushing with an accupipette. Trypsin was not used in the harvest
- Cell counting and survival determination with Trypan blue vital dye
- Preparation of slides: After harvest the cells were suspended in PBS (without CA++ and Mg++). A 10-µl Alliquot of cell suspension was mixed with 75 µl of 0.5% low-melting agarose and spread on a microscope slide covered with 1.0% normal-melting agarose. The slides were kept on ice for 5 min after which the coverslips were removed. The cells were treate with a lysing solution (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris, pH10, 1% sodium lauryl sarcosinate, 1 % Triton X-100) for 1 hr. at 4°C.
- Electrophoresis: The slides were placed in a horizontal electrophoresis tank and the DNA was allowed to unwind for 15 min in the electrophoresis buffer (1mM EDTA and 300 mM Na=H, pH > 13) before the run form 10 min at 25 V/300 mA. Slides of MCA and the concurrent solvent and positive control were run simultaneously in one elcetrophoresis tank. The slides were than neutralized with Tris buffer (0.4 M, pH 7.5).
-Single cell gel (SCG) analysis: In ethidium bromide-stained, coded slides (in 100 cells per dose, 50 cells per culture) using an automated image analysis ssystem (Komet 4.0.2; Kinetic Imaging, UK). Generally the comet and camera options recommended by the manufacturer were followed. The same settings were used in all experiments. As a hole the assay was set up to detect migration among the control cells without its being excessive.
- Parameters: Tail DNA (tail % DNA), tail extent moment [(tail
{[(tail mean -
}, and tail length.

STATISTICAL ANALYSIS: T-test;
level of significance: P < 0.05, P < 0.01, P < 0.001

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments

Flag: Critical study for SIDS endpoint

05-JAN-2004 (82)

Type: Sister chromatid exchange assay
System of testing: Chinese hamster ovary (CHO) cells
Concentration: first experiment: 0; 1.8; 3.6; 7.1 µM corresponding to 0; 0.30; 0.61; 1.20 µg/ml second experiment: 0; 1.5; 2.9; 5.9; 8.9 µM corresponding to 0; 0.25; 0.49; 1.0; 1.50 µg/ml
Cytotoxic Concentration: tested up to toxic concentrations as determined by a decrease of metaphases or in the frequency of second-division cells on the slides
Metabolic activation: without
Result: positive

Method: other: Method in general accordance with OECD method 479; however testing only without metabolic activation
Year: 2001
GLP: no
Test substance: other TS: Mucochloric acid, purity: 99% (source

Sigma-Aldrich); see 1.1.1

Remark: Comparative study of chlorohydroxyfuranones including MCA and MX

Result: SISTER CHROMATID EXCHANGE/CELL:
(* p<0.05, **p < 0.01, *** p < 0.01 vs solvent control, two tailed t-test.

MCA:
Experiment 1:
7.80; 8.00; 8.63; 8.83 at 0; 0.30; 0.61; 1.20 µg/ml
Positive control: 16.03***; 20.27*** at 10.02; 20.04 µg/ml

Experiment 2:
8.53; 8.63; 8.40; 9.50; 11.10** at 0; 0.25; 0.49; 1.0; 1.50 µg/ml
Positive control: 11.07***; 15.23*** at 5.01; 9.90 µg/ml

MX:
8.80; 9.80; 11.53**; 15.97***; 21.80*** at 0; 0.13; 0.27; 0.50; 0.74 µg/ml
Positive control: 11.07*; 15.70*** at 5.01; 9.90 µg/ml

Test condition: METABOLIC ACTIVATION SYSTEM: presumably without, but not explicitely stated
SOLVENT: PBS
CONTROLS: negative (solvent control: PBS) and positive control (Methyl methanosulfonate (MMS))
MEDIA:
MEM-alpha medium, supplemented with 10% fetal calf serum, penicillin (100 IU/ml, and streptomycin 100 µg/ml)
SELECTION OF TEST SUBSTANCE CONCENTRATIONS: up to toxic concentrations as determined by a decrease of metaphases or in the frequency of second division cells on the slides as determined in a pilot assay

NUMBER OF REPLICATES: 2 (dublicate flasks per dose group)

PERFORMANCE OF TEST:
- Preincubation of 2.5 x 10E5 CHO cells in 25 cm^2 flasks in dublicate, 24 hours before treatment.
- Exposure to TS or controls (50 µl) for 1 hour in PBS with CA++ and Mg++
- Cell-Incubation: further incubation for 25 hours with 5-bromodeoxyuridine (10µM) presumably in MEM-alpha medium but not explecitely stated
- Harvesting of cells: Addition of 2 x 10E-7 M Colcemid 2.5 hours prior to harvest, the cells were collected by shaking and treated with hypotonic solution (0.2 g KCl and 0.2 g sodium citrate in 100 ml of deionized water) at 37°C for 15 minutes. Fixation of cells three times with methanol : acetic acid (3:1 v/v). Dropping of fixed cells onto slides and air drying.
- Staining of cells: fluorescence plus Giemsa technique
- Analysis of slides: After coding the slides were analyzed by one observer; 30 harlequin-stained metaphases per dose respectively 15 harlequin-stained metaphases for the positive control

STATISTICAL ANALYSIS: T-test;
level of significance: P < 0.05, P < 0.01, P < 0.001

Reliability: (2) valid with restrictions

Study meets generally accepted scientific standards, well documented and acceptable for assessments
Flag: Critical study for SIDS endpoint
23-JUN-2003 (82)

Type: Chromosomal aberration test
System of testing: Chinese hamster ovary (CHO) cells
Concentration: 0; 1.5; 2.9; 5.9; 11.8; 14.8 µM corresponding to 0.25; 0.49; 1,00; 1,99; 2.50 µg/ml
Cytotoxic Concentration: 14.8 µM = 2.5 µg/ml
Metabolic activation: without
Result: positive

Method: other: Method in general accordance with OECD method 473; however testing only without metabolic activation; unclear which level of cytotoxicity is reached in the highest concetration
Year: 2001
GLP: no
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Remark: omparative study of chlorohydroxyfuranones including MCA and MX
Result: STATISTICAL SIGNIFICANCE LEVELS: * p<0.05, **p < 0.01, *** p < 0.01 vs solvent control, two tailed t-test.

MCA at 0.25; 0.49; 1,00; 1,99; 2.50 µg/ml:

CELLS WITH ABERRATIONS (%):
TOTAL:
Without gaps: 1; 2; 2; 4; 48***; 74***,
with gaps: 4; 5; 5; 7; 54***; 80***
Positive control:
Without gaps: 24***; 50***, with gaps: 26***; 54*** at 100; 150 µg/ml

CHROMATID TYPE ABERRATIONS:
Gaps: 3; 3; 3; 4; 12; 24, Breaks: 0; 2; 0; 3; 30; 34,
Exchanges: 0; 0; 0; 0; 36; 40
Positive control:
Gaps: 2; 12, Breaks: 10; 16, Exchanges: 9; 36 at 100; 150 µg/ml

CHROMOSOME TYPE ABERRATIONS:
Breaks: 0; 2; 5; 5; 4; 18, Exchanges: 1; 0; 0; 0; 0; 2
Positive control:
Breaks: 9; 4, Exchanges: 0; 2 at 100; 150 µg/ml

UNCLASSIFIED CELLS (cells that were too severely damaged to be classified, were included in the frequency of total aberrations:
0; 0; 0; 0; 0; 10
Positive control:
0; 6 at 100; 150 µg/ml

MX at 0; 0.24; 0.50; 1.00; 2.00 µg/ml (at 2.00 µg/ml only 50 cells analyzed):

CELLS WITH ABERRATIONS (%):

TOTAL:
Without gaps: 1; 1; 8*; 31***; 100***
with gaps: 2; 2; 9*; 33***; 100 ***
Positive control:
Without gaps: 21***; 64***, with gaps: 26***; 66*** at 100; 150 µg/ml

CHROMATID TYPE ABERRATIONS:
Gaps: 1; 1; 1; 6; 6, Breaks: 0; 0; 3; 12; 20, Exchanges: 0; 0; 0; 0; 17; 42
Positive control:
Gaps: 6; 4, Breaks: 9; 30, Exchanges: 7; 38 at 100; 150 µg/ml

CHROMOSOME TYPE ABERRATIONS:
Breaks: 1; 1; 4; 6; 6, Exchanges: 0; 0; 0; 1; 0
Positive control:
Breaks: 6; 12, Exchanges: 0; 0 at 100; 150 µg/ml

UNCLASSIFIED CELLS (cells that were too severely damaged to be classified, were included in the frequency of total aberrations:
0; 0; 0; 0; 3; 58
Positive control:
1; 2 at 100; 150 µg/ml

Test condition: TEST SUBSTANCES:
MCA
MX:
- Purity (NMR): >= 98%
- Concentrations: 0; 1.1; 2.3; 4.6; 9.2 µM corresponding to 0; 0.24; 0.50; 1.00; 2.00 µg/ml; 2.00 µg/ml cytotoxic concentration
POSITIVE CONTROL:
- Methyl methanesulfonate (MMS)
- Concentration: 908; 1362 µM corresponding to 100; 150 µg/ml

METABOLIC ACTIVATION SYSTEM: presumably without, but not explicitely stated
SOLVENT: PBS
CONTROLS: negative (solvent control: PBS, no untreated control) and positive
control (Methyl methanosulfonate (MMS))
MEDIA:
MEM-alpha medium, supplemented with 10% fetal calf serum, penicillin (100 IU/ml, and streptomycin 100 µg/ml)
SELECTION OF TEST SUBSTANCE CONCENTRATIONS: up to toxic concentrations as determined by a decrease of metaphases on the slides as determined in a pilot assay, no data on cytotoxicity parameters given

NUMBER OF REPLICATES: 1 (dublicate flasks per dose group)

PERFORMANCE OF TEST:
- Preincubation of 2.5 x 10E5 CHO cells in 25 cm^2 flasks in dublicate, 24 hours before treatment.
- Exposure to TS or controls (50 µl) for 1 hour in PBS with CA++ and Mg++
- Cell-Incubation: further incubation for 21 hours 1 presumably in MEM-alpha medium but not explecitely stated
- Harvesting of cells: Addition of 2 x 10E-7 M Colcemid 2.5 hours prior to harvest, the cells were collected by shaking and treated with hypotonic solution (0.2 g KCl and 0.2 g

sodium citrate in 100 ml of deionized water) at 37°C for 15 minutes. Fixation of cells three times with methanol : acetic acid (3:1 v/v). Dropping of fixed cells onto slides and air drying.
- Staining of cells: 4% Giemsa (pH 7.0) for 5 min
- Analysis of slides: After coding the slides were analyzed by one observer; 30 harlequin-stained metaphases per dose respectively 15 harlequin-stained metaphases for the positive control

STATISTICAL ANALYSIS: X^2-test or when required Fisher's exact probability test;
level of significance: *P < 0.05, **P < 0.01, ***P < 0.001

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments
Flag: Critical study for SIDS endpoint
05-JAN-2004 (82)

Type: Ames test
System of testing: Salmonella typhimurium TA 100
Concentration: no information given
Cytotoxic Concentration: no information given
Metabolic activation: without
Result: positive

Method: other: no details on method given
Year: 1993
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Remark: Comparative study of chlorohydroxyfuranones including MCA and MX
Result: REVERTANTS/NMOL:
MCA 3.6
MX 5600

POSITIVE CONTROL NUMBER OF REVERTANTS:
1 µg: 500-600
5 µg: 1400-1600

BACKGROUND NUMBER OF REVERTANTS:
90-120
Test condition: DOSE LEVELS:
- MCA five dose levels not specified
- MX five dose levels not specified
- Two plates per dose level

SOLVENT: DMSO

MUTAGENICITY OF THE COMPOUNDS:
- expressed as revertant numbers per nanomole; calculated by least-squares regression analysis of the linear part of the dose-response curve

POSITIVE CONTROL:
- Sodium Azid
- Dose Levels: 1 µg and 5 µg

no further details given
Reliability: (2) valid with restrictions
Scientifically acceptable study despite of limited experimental details in documentation
28-JAN-2004 (83)

Type: Unscheduled DNA synthesis
System of testing: Hepatocyte primary culture from Fisher F344 rats
Concentration: 0; 10.24; 12.8; 16; 20; 25 µM corresponding to 0; 1.73; 2.16; 2.70; 3.38; 4.22 µg/ml
Cytotoxic Concentration: 25 µM = 4.22 µg/ml
Metabolic activation: without
Result: positive

Method: other: see freetext
Year: 1999
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Remark: Comparative investigation on MX and MCA
Result: MCA:
NNG: -0.56; 0.10; 1.43; 0.74; 3.87; 5.11 at 0; 1.73; 2.16; 2.70; 3.38; 4.22 µg/ml; Positive control: 32.25 at 1.45 µg/ml
Cells in repair (%) (NNG > 5): 8; 15; 24.3; 15.7; 36.3; 49 at 0; 1.73; 2.16; 2.70; 3.38; 4.22 µg/ml; Positive control: 100 at 1.45 µg/ml
Survival of control: 100; 98.5; 99; 76.7; 38.3; 5.9 at 0; 1.73; 2.16; 2.70; 3.38; 4.22 µg/ml (4,22 µg/ml cytotoxic concentration)
No. of cells counted: 138; 146; 148; 140; 165; 148 at 0; 1.73; 2.16; 2.70; 3.38; 4.22 µg/ml; Positive Control: 148
No or cells in S phase: 0; 0; 1; 1; 0; 0 at 0; 1.73; 2.16; 2.70; 3.38; 4.22 µg/ml; Positive Control: 10 at 1.45 µg/ml
Conclusion: Significant genotoxic effect at 2.16; 2.70; 3.38 µg/ml; Postitive Control: Significant genotoxic effect at 1.45 µg/ml

MX:
NNG: -2.42; -1.37; -0.75; 0.33; 1.11; 5.49; 16.98 at 0; 1.61; 2.41; 3.62; 5.44; 8.15; 12.22 µg/ml; Positive control: 20.75 at 1.45 µg/ml
Cells in repair (%) (NNG > 5): 12; 15.7; 17.7; 25.3; 33.3; 50; 98 at at 0; 1.61; 2.41; 3.62; 5.44; 8.15; 12.22 µg/ml; Positive control: 94.7 at 1.45 µg/ml
Survival of control: 100; 93.2; 93.7; 97.5; 93.2; 95.6; 72.6 at at 0; 1.61; 2.41; 3.62; 5.44; 8.15; 12.22 µg/ml
No. of cells counted: 157; 142; 154; 156; 168; 154; 150 at at 0; 1.61; 2.41; 3.62; 5.44; 8.15; 12.22 µg/ml; Positive Control: 189 at 1.45 µg/ml
No or cells in S phase: 0; 0; 0; 0; 0; 0; 0 at 0; 1.61; 2.41; 3.62; 5.44; 8.15; 12.22 µg/ml; Positive Control: 1 at 1.45 µg/ml
Conclusion: Significant genotoxic effect at 3.62; 5.44; 8.15; 12.22 µg/ml; Postitive Control: Significant genotoxic effect at 1.45 µg/ml
Test condition: TEST SUBSTANCES:
MCA

MX:

- Purity >= 98% as determined bei 1H NMR and GC
- Concentrations: 0; 7.4; 11.1; 16.67; 25; 37.5; 56.25 µM corresponding to 0; 1.61; 2.41; 3.62; 5.44; 8.15; 12.22 µg/ml
AAF:
- Purity >=95% from Sigma
- Concentration: 6.5 µM corresponding to 1.45 µg/ml

HEPATOCYTE CULTURE:
- Perfusion of the liver of anesthetized male Fisher F334 rats with
a) HEPES buffer
b) HEPES/collagenase buffer
- Centrifugation of the cell suspension obtained (1 min at 40 g)
- Resuspension in medium
- Medium: William E mdium suplemented with 200 U/ml penicillin, 50 µg/ml streptomycin, 2.5 g/ml amphtericin B, 200 µg/ml L-glutamin and 10% (v/v) heat-inactivated fetal calf serum (WE-C)
- Determination of % of viable cells: trypan blue technique and a Malassez haemocytometer; criteria > 50% viable cells in final cell suspension
- Cell concentration: 1.5 x 10E5 viable cells/ml distributed in 6-well microplates containing round plastic coverslips
- Incubation for cell attachment: ca. 90 min at 37°C in 5% CO2 atmosphere

TREATMENT AND RADIOLABELLING OF HEPATOCYTE CULTURE:
- Suckling of medium and washing with medium as given above but without fetal calf serum (WE-I)
- Replacement by WE-I containing 10 µCi/ml [3H]thymidine and the test compound
- Concentrations
MCA: 10.24; 12.8; 16; 20; 25 µM corresponding to 1.73; 2.16; 2.70; 3.38; 4.22 µg/ml
MX: 7.4; 11.1; 16.67; 25; 37.5; 56.25 µM corresponding to 1.61; 2.41; 3.62; 5.44; 8.15; 12.23 µg/ml
- Negative Control (Solvent control): WE-I medium
- Positive Control: 6.5 µM AAF corresponding to 1.45 µg/ml
- cultures for determination of survival were treated simalar except without 3H-thymidine in medium

DETERMINATION OF SURVIVAL:
- determination at each concentration tested
- expression as percentage of solvent control
- highest concentration tested had usually between 50-75% survival in comparison with solvent control

AUTORADIOGRAPHIE:
- Preparation of slides: glewing of the cover-slips on normal microscopic slides; coating with Kodak D19 liquid emulsion; air-drying
- Incubation in a light tight box in the refrigerator for 10-14 days
- Development and fixation of the film emulsion
- Staining of cell nuclei and cytoplasm with Meyers hemalun
- Dehydration of slides in ethanol
- Cleaning with xylene and mounting of slides with coverslips for microscopic examination

AUTORADIOGRAPHIC ANALYSIS AND GRAIN COUNTING:
- Image analysis system Visilog
- 50 cells per slide and 3 slides per concentration; every cell recorded only once
- Only cells with normal morphology were scored; isolated nuclei without surrounding cytoplasm, cells with unusual staining or heavily labelled cells in S phase were not scored.
- Recording of nuclear grain counts (NC) and cytoplasmic grain counts (CC)
- Net nuclear grain per cell (NNG) = NC - CC

EXPRESSION OF RESULTS AND CRITERIA FOR GENOTOXIC ACTIVITY
- For each slide and concentration calculation of: average NNG; percentage of cells in repair; average CC; average NC; numer of cells in S phaese
- Statistics: non-parametric U rank Mann-Whitney test
- Compound considered genotoxic if :
a) at any concentration tested group mean value > 0 NNG and 20% or more of cells are in repair (NNG values > 5)
b) compared with control an increase is observed in both NNG and the percentage of cells in repair
c) a dose-related increase is seen both in NNG and in percentage of cells in repair

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP.

Flag: Critical study for SIDS endpoint

23-JUN-2003 (84)

Type: Micronucleus test in vitro
System of testing: L5178Y mouse lymphoma cells (strain TK+/- 3.7.2c)
Concentration: 1.56, 3.12, 6.25, 12.5, 25 µM i.e. 0.26, 0.52, 1.06, 2.11, 4.22 µg/ml
Cytotoxic Concentration: 4.22 µg/ml survival 35.3 % of control
Metabolic activation: without
Result: positive

Method: other: according to Nesslany and Marzin (1999) Mutagen 14: 403-410
Year: 1999
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Remark: Comparative investigation on MX and MCA
Result: MCA:
- Micronuclei/1000 mononucleated cells (mean of two tests): 5.5; 4; 3.5; 6; 9.5; 30** at 0.26, 0.52, 1.06, 2.11, 4.22 µg/ml
- Survival (% of control): 86.2; 93.1; 75.7; 70.5; 35.3 at 0.26, 0.52, 1.06, 2.11, 4.22 µg/ml

MX:
- Micronuclei/1000 mononucleated cells (mean of two tests): 4; 9; 7; 11.5*; 52.5** at 1.36; 2.71; 5.44; 10.87; 21.74 µg/ml
- Survival (% of control): 87.1; 80.6; 86.4; 76.6; 61.8 at

1.36; 2.71; 5.44; 10.87; 21.74 µg/ml

Positive Control (Mitomycin C):
- Micronuclei/1000 mononucleated cells (mean of two tests): 116.5** at 25 ng/ml

Statistical significancy levels:
* p<0.05; ** p<0.001

Test condition: CELLS:
- L5178Y mouse lymphoma cells (strain TK+/- 3.7.2.c)
- Storage frozen in aliquots per experiment
- Media: FM 10 medium i.e. Fisher medium supplemented with 200 U/ml penicillin, 50 µg/ml streptomycin, 2.5 µg/ml amphotericin B, 200 µg/ml L-glutamine, 200 µg/ml sodium pyruvate, 500 µg/ml pluronic acid and 10% (v/v) heat-inactivated horse-serum.
- Incubation: at 37°C in humified atmosphere containing 5% CO2
- Batch control: for absence of mycoplasma contamination
- Treatment of the batch with methotrexate to prevent the presence of spontaneous TK-/- mutants

CELL TREATMENT:
- Without metabolic activation
- Incubation 0.1 ml of exponentially growing cells (4 x 10E5 cells/ml) in a 96-well V-bottom microplate with FM 10 medium containing the test compound; without cytochalasin B (that was shown in preliminary experiments to induce DNA fragmentation and pycnotic nuclei)
- Treatment for 24 hours in dublicates
- Concentrations:
highest concentration should induce a significant reduction in MTT incorporation (cytotoxicity assay)
MCA: 1.56, 3.12, 6.25, 12.5, 25 µM i.e. 0.26, 0.52, 1.06, 2.11, 4.22 µg/ml
MX: 6.25; 12.5; 25; 50; 100 µM i.e. 1.36; 2.71; 5.44; 10.87; 21.74 µg/ml
- Centrifugation of microplates for 5 min at 900 r.p.m and discarding of the supernatant by gentle pouring off
- Further cell incubation with medium for 20 hours
- Parallel performance of cytotoxicity assay (see below)
- Harvesting of cells: Washing with 0.2 ml Fisher medium with 0.1% pluronic acid; gentle resuspension; Hypotonic treatment for 4 min with 0.2 ml Fisher medium : distilled water (1:1) + 0.1% pluronic acid; Fixation by addition of 0.1 ml of ethanol: acetic acid (3:1 v/v) for at least 10 min
- Positive Control: Mitomycin C 25 ng/ml
Slide preparation:
- Final resuspension by drawing and expelling using a Pasteur pipette; dropping onto clean glass slides and allowing to dry at room temperature for 24 hours
- Staining: 10 min in 2% Giemsa water solution
- Rinsing and Coding before analysis

ANALYSIS:
- under microscope 500 x magnification by two scorers; one for each series of slides
- Analysis of micronuclei in at least 1000 mononucleated cells per culture in two parallel cultures; i.e. 2000 cells per dose
- Determination of micronuclei according to criteria described by Miller et al. (1995) Environ Mol Mutagen 26: 240-247
- Criteria for positive results: a concentration related increase in the number of micronucleated cells and a

statistically significant increase over the spontaneous level in at least one treatment

STATISTICS:
- Significant differences between groups using the Chi²-test

CYTOTOXICITY ASSAY:
- MTT colourimetric method (Borenfreund et al 1988. Toxicol in vitro 2: 1-6)

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP.

Flag: Critical study for SIDS endpoint

23-JUN-2003 (84)

Type: other: PARP activation assay
System of testing: Chinese hamster ovary cells (XRCC1-proficient and -deficient)
Concentration: no data
Metabolic activation: without
Result: positive

Method: other: indirect assay for poly(ADP-ribose) polymerase (PARP) activation (formation of intracellular NAD(P)H)
Year: 2003
GLP: no data
Test substance: no data

Result: XRCC1-deficient CHO cells contained significantly less NAD(P)H than -proficient cells after incubation with MCA

After co-incubation of XRCC1-deficient cells with MCA and PARP inhibitor the decrease in intracellular NAD(P)H was almost completely blocked indicating that the decrease was primarily due to PARP activation throught formation of single strand breaks

Test condition: Slot blot assay which indirectly monitors the status of single strand break repair enzymes by circuitously measuring poly(ADP-ribose) polymerase activation through depletion of intracellular NAD(P)H

- Incubation of XRCC1-deficient and -proficient CHO cells for 4 hours with MCA
- Monitoring of NAD(P)H concentrations in living cells by incubation with a water soluble tetrazolium salt.
- Determination of the tetrazolium salt reduction to a yellow dye by spectrophotometric measurement
- Distinguishing whether NAD(P)H reduction was due to decreased mitochondrial function or NAD+ depletion by PARP activation by co-exposure of XRCC1-deficient cells to MCA and a PARP inhibitor

Reliability: (4) not assignable
Abstract

02-JUL-2003 (85)

Type: other: DNA aduct formation of calf thymus DNA with MCA in vitro (adenine adducts)
System of testing: Salmonella typhimurium: TA 100
Metabolic activation: without

Method: other: see freetext
Year: 1997
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Result: Identification of adducts formed in reactions of calf thymus DNA adenine with MCA:
- adduct:
3-(2´-deoxyribofuranosyl)-7-formylimidazo[2,1-i]purine
- yield: 5 adducts/10E6 nucleotides

Test condition: Reactions with calf thymus DNA
- 18.25 mg was reacted with double-stranded calf thymus DNA (3.75 mg) in 1.5 ml of 0.1 M phophate buffer at pH 6.5.
- mixture was stirred and incubated at 37°C for 2 and 4 days
- Monitoring and readjustment of pH during first 12 hours and than twice a day
- DNA recovery by precipitation with ethanol: incubation mixture with 0.2 ml of 5 M NaCl and 3 ml of cold 96% ethanol
- Centrifugation: 10 min at 3000 rpm; removal of supernatant
- Twice repeating of precipitation and centrifugation

Enzymatic hydrolysis of DNA:
- Dissolving of DNA in 3.75 ml of 0.1 M phophate buffer pH 7.4 containing 5 mM MgCl2
- Addition of DNase I (dissolved at 10 mg 0f DNase/ml in 0.9% NaCl) to obtain 0.1 mg of DNase/ml
- Incubation and stirring for 3 h at 37°C
- Addition of Nuclease P1 (dissolved at 0.5 mg/ml in mM ZnCl2) to obtain a final concentration of 20 µg nuclease/ml
- Addition of alkaline phosphatase (87 U/ml in water) and acid phosphatase (20 U/ml in water) to obtain final concentrations of 0.5 and 0.3 U/ml respectively
- Incubation and stirring for 18h at 37°C
- Rotary evaporation of hydrolyzed DNA to near dryeness
- Washing: four times 2.5 ml ethanol/methanol 1:1
- Combination of washes and removal of insoluble particles by centrifugation (20 min, 3000 rpm)
- Rotary evaporation of hydrolyzed DNA to near dryeness
- Addition of 0.1 ml water
- HPLC analysis of 20 µl injectate
- Additionally analysis of insoluble particles dissolved in water

HPLC analysis:
- Kontron liquid chromatographic system: model 322 pump; 440 diode array detector (UV); JASCO FP-920 fluorescence detector; KromaSystem 2000 data handling program
- column: C18 Sperisorb ODS2 analytical column 5 µm (4 x 125 mm); C8 Lichorspher 100 RP-8 column 5 µ (4 x 125 mm); C18 Sperisorb ODS2 analytical column 5 µm (4 x 250 mm)
- Elution: isocratically for 5 min with 5% acetonitrile in water followed by a gradient from 5% to 30% acetonitrile in 25 min at a flow rate of 1 mL/min

Preparative isolation:
- by Column chromatography
- Column: C18 Bondesil bound silica grade 40 µm (2.5 x 10 cm)
- purification on HPLC: Shimadzu LC-9A pumps, variable wavelength Shimadzu SPD 6A UV spectrophotometric detecdtor;

Rheodyne injector model 7120 equipped with a 2000 µL loop; injection volume 1ml; column: C18 analytical column (4 x 125 mm)

Spectroscopic and Spectrophotometric methods:
1H NMR spectra:
- JEOL JNM-A500 Fourier transform NMR spectrometer at 500 MHz
- samples dissolved in Me2SO-d6
- internal standard TMS
- determination of shifts and coupling constants in ribosyl units was based on first-order approach
UV-spectra and fluorescence spectra:
- as peaks eluted from the HPLC columns
Mass spectra:
- Fisions ZABSpec-oaTOF instrument
- Ionisation mode: either electron impact or electrospray
- Electron impact: at 70 eV; samples applied through direct inlet probe
- Electrospray: using nitrogen as both nebulizing and bath gas; potential of 8.0 kV applied to the needle;
- temperature of pepperpot counter electrode 90°C;
- sample introduction by loop injection at flow rate of 20 µl/min (H2O/CH3CN/acetic acid: 80/20/1)
- standards: PFK and PEG 200
- resolution of a mass spectrometer: 7000
(1H-NMR-, 13C-NMR-, MS-, UV- spectra)

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; no guideline study; in vitro results

Flag: Critical study for SIDS endpoint

05-JAN-2004 (86)

Type: other: chiral recognition of mutagenicity in the Ames test; DNA adduct formation
System of testing: Salmonella typhimurium TA 100
Metabolic activation: without

Method: other: see freetext
Year: 1993
GLP: no data
Test substance: other TS: Mucochloric acid, technical grade, 99% purity

Result: Mutagenicity of MCA and adducts from MCA and MCA-cysteine adducts given as molar mutagenicity (4 resp. 3 experiments per substance):

MCA: 2,340: 2,050; 1,870; 1,810 revertants/µmol; mean 2,020 revertants/µmol corresponding to 13.8; 12.1; 11.1; 10.7 revertants/µg; mean 12.0 revertants/µg

MCA-(R)-(+)-cysteine: 3.92; 9.56; 3.13; 5.26 revertants/µmol; mean 5.47 revertants/µmol

MCA-(S)-(-)-cysteine: 3.96; 6.37; 4.19; 5.54 revertants/µmol; mean 5.02 revertants/µmol

MCA-(R,S)-(+/-)-cysteine: 2.66; 4.83; 3.43 revertants/µmol; mean 3.64 revertants/µmol

Test condition: MUTAGENICITY ASSAY:
- Strain: TA100

- Standard plate incorporation assay
- Without metabolic activation
- Method according to Maron and Ames (1983) Mutat Res 113: 173-215
- Solvent: DMSO
- Three plates per dose level
- Zero dose: Solvent DMSO
- Controls (five plates per control): solvent control (DMSO); crystal violet; ampicillin; sodium azide

- Mutagenicity values as revertants per µg obtained from positive linear regression slopes of the ascending portion of the curve extending to the maximum value of revertants as determined by the statistical treatment of Bernstein et al. (1982) Mutat Res 97: 267-281.
- Statistical Significance: Difference of group means by t-test or ANOVA at the 95% level
- Calculation of the molar mutagenicity

CHIRAL TEST SUBSTANCES:
- MCA-(R)-(+)-cysteine; purity 99%
- MCA-(S)-(-)-cysteine; purity 99%
- MCA-(R,S)-(+/-)-cysteine; purity 99%

SPECTRA AND ELEMENTAL ANALYSES:
1H-NMR, 13C-NMR and 2D NMR:
- Brucker AMX 300 spectrometer
- 1H-NMR at 300 MHz, 13C-NMR at 75.45 MHz
- Chemical shift values relative to tetramethylsilane (TMS) (sigma = 0.00 ppm)
- Determination of quarternary CH, CH2 or CH3 carbons achieved by distorionless enhancement by polarization transfer (DEPT) experiments
UV-spectra:
- Variant DMS 100 spectrophotometer
EIMS:
- Finnigan 4021 mass spectrometer
Optical rotations:
- Perkin Elmer 141 polarimeter
- using a 10 cm path-length cell
Circular Dichroism (CD)
- Jasco Model ORD/UV5 modified for CD by Sproul Scientific part number SS-107
- determined in methanol solution
Elemental analysis
- performed by Desert Analytics

X-Ray Analysis
- X-ray structure of racemic form MCA-(R,S)-(+/-)-cystein determined by PJ Caroll (Chemistry Department University of Pennsylvania, Philadelphia)

Conclusion: Based on data of this study and on previous data (see LaLonde and Xie (1992) Chem Res Toxicol 5: 618-624)
MCA-(R)-(+)-cysteine is considered to be 2 to 4 times more mutagenic than MCA. No enantiospecific interaction between enantiomers and chiral DNA or enzymes involved in repair and replication could be concluded

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments

Flag: Critical study for SIDS endpoint

06-MAY-2004 (87)

Type: other: Mutagenicity of reaction products in vitro (adduct formation with cysteine and acetylcysteine-conjugation)
System of testing: Salmonella typhimurium TA 100
Metabolic activation: without

Method: other: see freetext
Year: 1992
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Result: Inactivation of mutagenicity by reaction of MCA with NCA:
- after 6 h only 53% of initial mutagenicity
Kinetics of inactivation:
-second order rate constant at initial concentration ratios of MCA/NCA 2:1, 1:1, 1:2
Products:
- three products that resulted from the displacement of chlorine from C-3 or C-4 of MCA determined in levels of 7 (product 4), 2 (product 5) and 0.3% (product 6a)
- mutagenicity of products: product 4: nonmutagenic; product 5: weakly mutagenic, product 9a (product with chlorine conservation): comparable to MCA or more mutagenic than MCA

Test condition: Chemicals:
(R)-(+)-N-Acetylcystein (NCA) from Aldrich
(R)-(+)-Cysteine from Aldrich

Chromatography:
TLC:
- Merck silica gel 60F-254 sheets
Flash chromatography
- Merck Kieselgel 60 (230-400 mesh)
HPLC:
- Shimadzu LC-6A
- Column: ODS column (4.6 x 150 mm)
- Elution: idiocratically by 35% MeOH-water at ambient temperature at a flow rate of 1 ml/min
- detector wave length: 254 nm

Spectra and Elemental analysis:
NMR:
- Brucker AMX 300 spectrometer
- Standard for chemical shift values: TMS
- Dissolved in CDCl3 so

Reliability: (2) valid with restrictions
Scientifically acceptable study despite of limited experimental details in documentation
Flag: Critical study for SIDS endpoint

05-JAN-2004 (45)

Type: other: Mutagenicity of reaction products (Glutathion conjugation) in vitro
System of testing: Salmonella typhimurium: TA 100

Method: other: see freetext

Year: 1994
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Result: Reaction of MCA with Glutathion
- Components eluted in HPLC at 4.52, 6.74, 7.11 and 28.17 min retention time; in control experiment were peaks at 4.52, 6.74, 7.11 retention time absent
- peak at 4.52 min retention time = GSSG: 10.5% (1H NMR)
- peak at 6.74 and 7.11 min retention time GSH-MCA conjugates: 69.7% (HPLC)
- peak at 28.17 min retention time = MCA: 21.6% (HPLC)

- Discovery of GSSG as reaction product indicates and oxidation of GSH by MCA
- Reaction of MCA with GSH is accompanied by the formation of a radical species of MCA (EPR-analysis)
- it is unclear wether the MCA radical represents an intermediate leading eventually to nonmutagenic conjugates or whether it is involved in a separate shunt oxidation process depleting the reaction system of GSH that would otherwise be availabale for more efficient inactivation through the complete conjugation

Test condition: NMR Spectra and Chromatography:
1H NMR:
- in D20 at 300 MHz
- on a Bruker AMX 300 spectrometer
- Chemical shift values relative to TMS (sigma = 0.00 ppm)

HPLC:
- Shimadzu LC-6A
- at ambient temperature
- Column: Shimadzu ODS (150 x 4.6 mm)
- Isocratic elution with CH3CN/THF/H20 9:1:1 (pH 2.96)
- Flow rate 0.3 ml/min
- Detection wavelength: 254 nm

Reaction of MCA with GSH
- Mixture of 80 mg, 0.48 mmol MCA and 150 mg, 0.48 mmol GSH in 15 ml aqueous 0.1 M phophate buffer solution (K2HPO4/KH2PO4) at pH 7.0; buffer degassed for 6 h with a stream of N2
- Incubation for 24 h under N2
- Withdrawel of 1 µl portions with a syringe for HPLC analysis
- Component separation by eluent freeze-drying
- Dissolvance of powder in D2O for H NMR analysis
- Control experiment: same conditions but without MCA

EPR

- About 10 ml of 0.1 M sodium phosphate buffer (pH 7) was purged with N2 for at least 1 h
- Preparation of spin trap solution: Stirring 0.023M 2-methyl-2-nitrosopopane (tNB) in N2-purged buffer at 35 °C for 2 h
- Addition of MCA (0.032 M) and glutathion (0.030 M)
- Incubation under stirring in closed containers for 20 h at room temperature
- EPR-spectrometer: Bruker ESP300
- Recording of spectra at 9.77 GHz wit 100-kHz modulation frequency
- Each incubation sample was either pipetted or apirated into

a quartz flat cell centerd in an ER-4103 TM110 cavity
- Calibration of g-values of the radical adducts with a standard signal form Fremy's salt (g = 2.0057 +/- 0.0001)
- Computer simulation by laboratory intern software

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; no guideline study; in vitro results

Flag: Critical study for SIDS endpoint

05-JAN-2004 (46)

Type: other: Mutagenicity of reaction products in vitro (glutathion conjugation)
System of testing: Salmonella typhimurium: TA 100
Metabolic activation: without

Method: other: see freetext
Year: 1993
GLP: no data
Test substance: other TS: Mucochloric acid, technical grade, 99% purity

Result: REACTION PRODUCTS OF MCA WITH GSH:
- Formation of a mixture of two diastomers resulting from displacement of the C-4-Cl by the sulfur of GSH
- Ratio of diastomers: 1.5:1
- These two diasomers accounted for 70% of the product as determined by HPLC
- after recristalization the diastomeric product was 99% pure
- reaction of MCA with GSH without undergoing ring-chain tautomerism

Kinetics of MCA-GSH adduct formation at 25 °C:
- second order kinetcs for all three ratios tested MCA:GSH 1:1; 2:1; 1:2
- compared to reaction of MCA with N-acetylcysteine reaction of MCA with GSH is 5-6 times more reactive

Mutagenicity of MCA and MCA-GSH adduct

MCA: 2,130; 2,710; 2,310; 1,030 revertants/µmol; mean 2,800 revertants/µmol corresponding to 12.6; 16.0; 13.7; 23.9

revertants/µg; mean 16.6 revertants/µg

MCA-GSH: at lowest dose tested (20 resp. 50 µg/plate) increase of 30-40 revertants/plate relative to spontaneous revertants but no dose-dependent increase

Test condition: NMR Spectra and Chromatography:
TLC:
- Merck silica gel 60FG-254 sheets
- Solvent systems:
1H NMR, 13C NMR and 2D NMR:
- 1H NMR in D20 at 300 MHz
- 13C NMR at 75.45 MHz
- on a Bruker AMX 300 spectrometer
- Chemical shift values relative to TMS (sigma = 0.00 ppm)
- Determination of quarternary CH, CH2 or CH3 carbons achieved by distorionless enhancement by polarization transfer (DEPT) experiments
HPLC:
- Shimadzu LC-6A

- at ambient temperature
- Column: Shimadzu ODS (150 x 4.6 mm)
- Isocratic elution with CH3CN/THF/H20 9:1:1 (pH 2.96)
- Flow rate 0.3 ml/min
- Detection wavelength: 254 nm

UV Spectra:
- Kontron UVIKON 860 spectrophotometer

Optic rotation:
- Perkin Elmer 141 polarimeter

Elemental analyses:
- Performed by Desert Analytics

X-Ray structure analysis:
- determined by PJ Carroll (Chemistry Department, University of Pennsylvania, Philadelphia)

Circular Dichroism (CD)
- Jasco Model ORD/UV5 modified for CD by Sproul Scientific part number SS-107
- determined in methanol solution

Kinetics:
- Mixture of 15 µl of 0.037 M solutions of MCA and 7.5, 15 or 30 µl of 0.037 M solution of GSH in 0.1 M phosphate buffer (pH 7)
- Incubation in 1.0 cm sample cuvette containing 2 ml of buffer
- Immediate dilution with 3.5 ml of buffer
- Incubation in: cuvette holder at 25 °C
- Reference cuvette contains 0.1 M phosphate buffer
- Measuring of absorbance over the range from 200-400 nm over a period of 6 h.
- Kinetic data determined by decreasing absorbance at 261 nm (MCA) and increasing absorbance at 311 nm

Reaction of MCA with GSH

- Mixture of 40 mg, 0.24 mmol MCA and 74 mg, 0.24 mmol GSH in 8 ml aqueous 0.1 M phophate buffer solution (K2HPO4/KH2PO4) at pH 7.0
- Incubation at 37 °C under N2 over night
- Thereafter acification of the solution with 10% aqueous HCl
- Freeze-drying of aqueous phase
- Recrystalization from methanol-water of the freeze-dry residue

DETERMINATION OF MUTAGENICITY:
- Ames test according to Maron and Ames (1985) Mutat Res 113: 173-215
- Standard plate incorporation assay
- Tester strain Salmonella typhimurium TA 100 without S9-Mix
- Testing of MCA and the reaction product dissolved in freshly prepared Me2SO4 solution added to the top agar
- Three plates per dose level
- Zero dose: Solvent Me2SO4 (five plates per control)
- Controls (five plates per control): solvent control (Me2SO4); crystal violet; ampicillin; sodium azide
- Mutagenicity values as revertants per µg obtained from positive linear regression slopes of the ascending portion of the curve extending to the maximum value of revertants as determined by the statistical treatment of Bernstein et al. (1982) Mutat Res 97: 267-281.
- Calculation of the molar mutagenicity

Conclusion: Loss of mutagenicity of MCA by GSH conjugation:
MCA-GSH not mutagenic in Ames test TA 100 tester strain
GSH is more reactive and more specific for reacting with the closed ring form of MCA (without ring-chain tautomerism)
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; no guideline study; in vitro results
Flag: Critical study for SIDS endpoint
05-JAN-2004 (47)

Type: other: Reaction products MCA with adenosine in vitro

Method: other: see freetext
Year: 1995
GLP: no data
Test substance: other TS: Mucochloric acid, purity: >= 98% (source Fluka); see 1.1.1

Result: - Two peaks occuring at longer retention times were identified as
3-(beta-D-Ribofuranosyl)7-formyl-8-[9'-(beta-D-ribofuranosyl)-N8-adenonsinyl]imidazo[2,1-i]purine (epsiloncA,A) and
3-(beta-D-Ribofuranosyl)7-oxalo-8-[9'-(beta-D-ribofuranosyl)-N8-adenonsinyl]imidazo[2,1-i]purine (epsilonoA,A)
Test condition: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; no guideline study; in vitro results
Flag: Critical study for SIDS endpoint
05-JAN-2004 (88)

Type: other: Reaction products with adenosine and cytidine in vitro

Method: other: see freetext
Year: 1993
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Remark: The formation of the ethanocarbaldehyde derivatives and the previously identified etheno derivatives form mucochloric acid is explained by an initial conversion of mucochloric acid, through hydrolysis and decarboxylation to chloromalonaldehyde. Chloromalonaldehyde reacts with the nucleosides and forms an intermediate adduct which either undergoes ring closure by intramolecular displacement of the chlorine atom or breaks down to form chloroacetaldehyde which subsequently produces the etheno derivatives
Result: - Additional to the previous identified major product peak to be formed when MCA was reacted with adenosine or cytidine at 90°C pH 7 (see Kronberg L et al (1992) a second small peak was identified which eluted 4-5 min later
- Yield of this reaction product increased when reaction pH was 4.0
- peak was also identified in reaction of MCA with adenosine

at 37 °C pH 7.4 but not in reaction of MCA with cytidine

PRODUCT IDENTIFICATION:
- Reaction with adenosine: ethenoadenosinecarbaldehyde [3-(beta-D-Ribofuranosyl)-7-formylimidazo[2,1-i]purine9
- Reaction with cytidine: ethenocytidinecarbaldehyde [6-(beta-D-Ribofuranosyl)-7-formylimidazo[2,1-c]pyrimidin-5-(6 H)-one]

Test condition: ANALYSIS:
- Product isolation and sampling with HPLC
- HPLC1: Instrumed containing 2 Shimadzu LC-9A pumps and a Shimadzu SPD-6A UV spectrophotometric detector; detector at 290 nm
- HPLC2: HP 1090 equipped with diode-array detector
- Separation on Sperisorb ODS2 5 µm C18 reversed phase column (4 x 250 mm); isocratic elution for 5 min with 10% methanol in 0.01 M potassium dihydrogen phophate (pH 4.6) followed by a gradient from 10% to 30% methanol in 20 min at 1 ml/min
- Preparative isolation of products: on Nucleosil 7C18 semipreparative column 7 µm (10 x 250 mm); isocratic elution with 8% (adenosine reaction mixture) and 7.5 (cytidine reaction mixture) acetonitril in pure water
- 1HNMR and 13CNMR: JEOL GX-400 FT NMR spectrometer at 400 and 100 MHz respectively; samples dissolved in Me2SO4-d3; internal standard tetramethylsilane
- Assignment of carbon signals using proton-coupled and selectively proton-decoupled 13C NMR spectra
- Direct inlet electron impact (EI) mass spectra: VG 7070E mass spectrometer at 70 eV
- UV spectra: Shimadzu UV -160 spectrophotometer with diode array detector

REACTIONS WITH NUCLEOSIDES:
a) Reaction temperature: 90 °C for 24 h
- 8.2 mmol (1.38 g) MCA and 4.1 mmol (1.1 g) adenosine resp. 4.1 mmol (1.0 g) cytidine were added to 250 ml of a 0.5 M potassium phosphate buffer solution, adjusted to pH 4.0
- at the end of the reaction the reaction volume was reduced by rotary evaporation to approx. 80 ml
- reactions followed by HPLC separation and isolation
- collected fractions containing the products were rotary evaporated to dryness and residues subjected to spectrometric studies

b) Formation of etheno and ethnocarbaldhyde derivatives at various reaction conditions
- 0.08 mmol (13.1 mg) MCA was reacted with 0.04 mmol (10.7 mg) adenosine or 0.04 mmol (9.7 mg) cytidine in 2 ml of 0.5 M potassium phosphate buffer solutions
- reactions carried out at 90 °C at pH 4.0, 6.0 and 7.4 and at 37 °C at pH 7.4

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; no guideline study; in vitro results

Flag: Critical study for SIDS endpoint

05-JAN-2004 (89)

Type: other: Reaction products of MCA with adenosine, guanonsine and cytidine in vitro

Method: other: see freetext
Year: 1996
GLP: no data
Test substance: other TS: no data

Result: - Reaction of MCA and Mucobromic acids with adenosine and cytidine in DMF resulted major product peaks which were identified as chloro- or bromopropenal derivatives respectively.
- Prenal derivatives of MCA with adenosine and cytidine are formed in DMF (yields of 18.5 and 7.7% resp.) as well as to a much smaller amount in aqueous solutions (about 5 x 10E-3% each).

- The reaction mechanism was investigated by analysis of 13C-labelling, which showed that only the aldehyde carbon of the chloroprenal unit was labeled when reaction was performed in DMF while only the carbon in the formyl group was labeled in the aqueous reaction.
Reaction of MCA with guanosine: Formation of only trace levels of products that were not further investigated

Test condition: ANALYSIS:
- Product isolation and sampling with HPLC
- HPLC1: Instrument containing 2 Shimadzu LC-9A pumps and a Shimadzu SPD-6A UV spectrophotometric detector; detector at 290 nm
- HPLC2: HP 1090 equipped with diode-array detector
- Separation on Spherisorb ODS2 C18 5µm (4 x 125 mm) analytical column; isocratic elution for 5 min with 5% acetonitrile in 0.01 M potassium dihydrogen phosphate (pH4.6) followed by a gradient from 5 to 30% in 25 at a flow rate of 1 ml/min
- Preparative isolation of products by column chromatography on a 2.5 x 10 cm column of preparative C18 bond silica grade (40 µm, Bondesil); equilibration with water followed by batchwise elution with 0%, 5%, 10% and 15% acetonitrile in water batch volume: 100 ml
- 1HNMR and 13CNMR: JEOL JNM-A500 Fourier transform NMR spectrometer at 500 and 125 MHz respectively; samples dissolved in Me2S04-d6; internal standard tetramethylsilane
- Determination of shifts and coupling constants of the multiplets of the proton signals in the ribose units of adenosine adducts based on first order approach; for cytidine adducts due to small shift differences and interproton couplings calculation of spectral parameters unsing PERCH program
- Direct chemical ionization (DCI) mass spectra: VG 7070E mass spectrometer; ionization gas methane
- UV spectra: Shimadzu UV -160 spectrophotometer

PREPARATION AND PURIFICATION OF 13C-MCA:
- according to the method of Franzén and Kronberg (1995) Tetrahedron Lett 36:3905-3908

REACTIONS WITH NUCLEOSIDES:

a) Reaction with adenosine
- 1.19 mmol (200 mg) MCA was reacted with 0.59 mmol (159 mg) adenonsine in 8 ml of DMF for 3 days at 37 °C
- alternatively 1.19 mmol (200 mg) 13C-MCA (15 mol%) was reacted with 0.59 mmol (159 mg) adenonsine in 8 ml of DMF for

3 days at 37 °C

b) Reaction with cytidine
- 1.19 mmol (200 mg) MCA was reacted with 0.59 mmol (143 mg) cytidine in 20 ml of DMF for 5 days at 37 °C

c) Reaction with guanosine
- 1.19 mmol (200 mg) MCA was reacted with 0.59 mmol guanosine in 20 ml of DMF for 5 days at 37 °C

- after reactions solvent removal by rotary evaporation at 50 °C; residues dissolved in few ml water
- after filtering passage through the preparative C18 column

d) Small scale aqueous reaction with adenosine and cytidine
- 0.045 mmol (12 mg) adenosine resp. 0.045 mmol (11 mg) cytidine were reacted with 0.09 mmol (15 mg) MCA each in 2 ml of 0.5M phosphate buffer at pH 7.4 and 6.0 at 37 °C for 5 days
- determination of product formation by HPLC analysis of aliquots of the reaction mixtures

e) Aqueous reaction of 13C-3 labeled MCA with adenosine
- 0.32 mmol (85 mg) adenosine was reacted with 0.61 mmol MCA mixed with 13C-MCA (102 mg in total; 13 mol% 13-C-MCA) in 0.5M phosphate buffer at pH 6.0 at 90 °C for 12 h
- After filtration isolation of the products by use of the preparative C18 column as described above
- Upon evaporation of the fractions containing the products the compounds were crystallized; recrystallization was performed from warm water

QUANTIFICATION OF PRODUCT YIELDS:
- Quantitative 1H NMR analysis using 1,1,1-trichloroethane as an internal standard was performed on aliquots of the adducts
- Preparation of standard solutions for HPLC analysis by taking of exact volumes of the NMR samples and diluting them with appropriate volumes of water
- Quantitative determination of adducts in the reaction mixtures by comparing the peak area of the adducts at 330 nm in the standard solution with the area of the adduct peak in the reaction mixtures
- Calculation of the molar yields from the original amounts of adenosine or cytidine in the reaciton mixture

Conclusion: Based on the result of this study the formation pathway suggested for etheno and ethenocarbaldehyde derivatives as described in Kronberg et al. (1992) Chem. Res. Toxicol. 5: 852-855 was revised by the authors
The now suggested pathway for the formation of the chloroprenal derivatives, ethanocarbaldehyde derivatives and etheno derivatives from mucochloric acid in aquesous solutions is explained by an initial formation of mucoxychloric acid, which may be further broken down to chloractaldehyde, which could proceed via the chloromalonaldehyde that reacts with the nucleosides and forms subsequently the derivatives.

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; no guideline study; in vitro results

Flag: Critical study for SIDS endpoint

05-JAN-2004 (90)

Type: other: Reaction products of MCA with adenosine, cytidine, guanosine and uridine

Method: other: see freetext
Year: 1992
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Result: Either at 90 °C or at 37 °C one major product peak

REACTION WITH CYTIDINE:
- at 90 °C MCA consumed after 24 h reaction time
- product peak 3-5 min later than unmodified nucleosides
- at 37 °C, pH 7.0 product peak at the same retention time after 7 days reaction time
- product peak at 37 and 90°C identical and identified as 3,N4-ethenocytidine
- additionally poorly retained hydrophoilic compounds were formed in the reactions, partly due to MCA degradation in water

REACTION WITH ADENOSINE:
- at 90 °C MCA consumed after 45 h reaction time
- product peak 3-5 min later than unmodified nucleosides
- at 37 °C, pH 7.0 product peak at the same retention time after 7 days reaction time
- product peak at 37 and 90°C identical and identified as 1,N6-ethenoadenosine
- additionally poorly retained hydrophoilic compounds were formed in the reactions, partly due to MCA degradation in water

REACTION WITH GUANOSINE:
- at 90 °C MCA consumed after 45 h reaction time
- product peak 3-5 min later than unmodified nucleosides
- at 37 °C, pH 7.0 product peak at the same retention time after 7 days reaction time
- product peak at 37 and 90°C identical and identified as 1,N2-ethenoguanosine
- additionally poorly retained hydrophoilic compounds were formed in the reactions, partly due to MCA degradation in water

REACTION WITH URIDINE:
- no observable reaction between MCA and uridine

Test condition: ANALYSIS:
- Product isolation and sampling with HPLC
- HPLC1: Instrument containing 2 Shimadzu LC-9A pumps and a Shimadzu SPD-6A UV spectrophotometric detector; detector at 290 nm
- HPLC2: HP 1090 equipped with diode-array detector
- Separation on C18 reversed phase columns
- 1HNMR and 13CNMR: JEOL GX-400 FT NMR spectrometer at 400 and 100 MHz respectively; samples dissolved in DMSO-d3 (containing a few percent of CDCl3); internal standard tetramethylsilane
- Homo- and heteronuclear shift correlation and NOE experiments: JEOL standard programs

- Direct chemical ionization (DCI) mass spectra: VG 7070E mass spectrometer; source temperature 200°C; emission current 0.5 mA; electron energy 100 eV; resolution 1000; ionization gas methane
- EI mass spectra: HP 5971A mass selective detector connected to a HP 5890 (series II) gas chromatograph
- UV spectra: Shimadzu UV -160 spectrophotometer

REACTIONS WITH NUCLEOSIDES:

a) Reaction temperature: 90 °C
- 8.75 mmol (1478.4 mg) MCA was added to 100 ml of a 0.05 M potassium phosphate buffer solution, pH 7 containing either 1,9 mmol cytidine, adenosine, guanosine or uridine.
- reactions followed by HPLC analysis: Column 7 µm (4 x 250 mm) Semipreparative Nucleosil 7 C18 column; separation: isocratic elution with 7% acetonitril in water

b) Reaction temperature: 37 °C
- 5 µmol (0.84 mg) MCA reacted with 0.5 µmol cytidine, adenosine, guanosine or uridine in 10 ml 0.05 M potassium phosphate buffer solution, pH 7
- reactions followed by HPLC analysis: Column 5 µm (4 x 250 mm) Spherisorb ODS2 C18 column;
- Separation: isocratic elution for 5 min with 5% acetonitrile in 0.05 M potassium dihydrogen phosphate (pH 4.6); followed by gradient from 5 to 15% acetonitrile in 15 min at 1 ml/min

Fractions:
- fractions containing product peaks were rotary evaporated to dyness; recristallization of the products from water (3,N4-ethenocytidine and 1,N3-ethenoguanosine) respectively from water/ethanol (1,N6-ethenoadenosine)

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; no guideline study; in vitro results

Flag: Critical study for SIDS endpoint

05-JAN-2004 (91)

Type: other: Mutagenicity of reaction products in vitro (adduct formation with cysteine)
System of testing: Salmonella typhimurium: TA 100
Metabolic activation: without

Method: other: see freetext
Year: 1993
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Result: Mutagenicity of MCA and adducts from MCA and MCA-cysteine adducts given as molar mutagenicity (4 resp. 3 experiments per substance):

MCA: 2,340: 2,050; 1,870; 1,810 revertants/µmol; mean 2,020 revertants/µmol corresponding to 13.8; 12.1; 11.1; 10.7 revertants/µg; mean 12.0 revertants/µg

MCA-(R)-(+)-cysteine: 3.92; 9.56; 3.13; 5.26 revertants/µmol;

mean 5.47 revertants/µmol

MCA-(S)-(-)-cysteine: 3.96; 6.37; 4.19; 5.54 revertants/µmol; mean 5.02 revertants/µmol

MCA-(R,S)-(+/-)-cysteine: 2.66; 4.83; 3.43 revertants/µmol; mean 3.64 revertants/µmol

Test condition: MUTAGENICITY ASSAY:
- Strain: TA100
- Standard plate incorporation assay
- Without metabolic activation
- Method according to Maron and Ames (1983) Mutat Res 113: 173-215
- Solvent: DMSO
- Three plates per dose level
- Zero dose: Solvent DMSO
- Controls (five plates per control): solvent control (DMSO); crystal violet; ampicillin; sodium azide
- Mutagenicity values as revertants per µg obtained from positive linear regression slopes of the ascending portion of the curve extending to the maximum value of revertants as determined by the statistical treatment of Bernstein et al. (1982) Mutat Res 97: 267-281.
- Statistical Significance: Difference of group means by t-test or ANOVA at the 95% level
- Calculation of the molar mutagenicity

CHIRAL TEST SUBSTANCES:
- MCA-(R)-(+)-cysteine; purity 99%
- MCA-(S)-(-)-cysteine; purity 99%
- MCA-(R,S)-(+/-)-cysteine; purity 99%

SPECTRA AND ELEMENTAL ANALYSES:

1H-NMR, 13C-NMR and 2D NMR:
- Brucker AMX 300 spectrometer
- 1H-NMR at 300 MHz, 13C-NMR at 75.45 MHz
- Chemical shift values relative to tetramethylsilane (TMS) (sigma = 0.00 ppm)
- Determination of quarternary CH, CH2 or CH3 carbons achieved by distorionless enhancement by polarization transfer (DEPT) experiments
UV-spectra:
- Variant DMS 100 spectrophotometer
EIMS:
- Finnigan 4021 mass spectrometer
Optical rotations:
- Perkin Elmer 141 polarimeter
- using a 10 cm path-length cell
Circular Dichroism (CD)
- Jasco Model ORD/UV5 modified for CD by Sproul Scientific part number SS-107
- determined in methanol solution
Elemental analysis
- performed by Desert Analytics

X-Ray Analysis
- X-ray structure of racemic form MCA-(R,S)-(+/-)-cystein determined by PJ Caroll (Chemistry Department University of Pennsylvania, Philadelphia)

Conclusion: Based on data of this study and on previous data (see LaLonde

and Xie (1992) Chem Res Toxicol 5: 618-624)
MCA-(R)-(+)-cysteine is considered to be 2 to 4 times more mutagenic than MCA. No enantiospecific interaction between enantiomers and chiral DNA or enzymes involved in repair and replication could be concluded.

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments

Flag: Critical study for SIDS endpoint

29-APR-2004 (87)

5.6 Genetic Toxicity 'in Vivo'

Type: other: Evaluation of nuclear anomalies in intestinal epithelial cells including micronuclei
Species: mouse **Sex:** male
Strain: B6C3F1
Route of admin.: gavage
Exposure period: single dose
Doses: 0, 0.23, 0.36 or 0.47 mmol/kg bw (ca. 0, 38.9, 60.8 or 79.4 mg/kg bw)

Method: other: Assay for micronuclei in tissues of GI tract
Year: 1991
GLP: no data
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Result: Mucochloric acid was considered by the authors only marginally positive with regard to induction of total nuclear anomalies. Total nuclear
anomalies (these included micronuclei, pyknotic nuclei, and karyorrhectic nuclei) were discussed by the authors to be only induced in the most
sensitive tissue, the duodenum. The increase was seen only statistically significant (P = 0.04) at the highest dose (0.46 mmol/kg = 79.4 mg/kg bw) and considered a "suggestive response". In contrast the tabulation of the study results states that the total nuclear anomalies induced in the intermediate (0.37 mmol/kg = 60.8 mg/kg bw) and high dose (0.46 mmol/kg = 79.4 mg/kg bw) are statistically significantly increased.

However within the total nuclear anomalies investigated only the micronuclei can be attributed directly to a mutagenic effect. Therefor only this parameter was evaluated in the light of in vivo genotoxicity.
It is given that 10% of the mice i.e. 1 of 10 per dose group for all three dose levels 0.23, 0.36 or 0.47 mmol/kg bw (38.9, 60.8 or 79.4 mg/kg bw) showed micronuclei. Therefore this study is considered to be equivocal with regard to in vivo genotoxicity.

For comparison: the structural analogue MX also induced dose-related changes in the proximal colon and the forestomach, which were statistically significant for the %age of animals with micronuclei at the highest dose level in the forestomach and duodenum (in 1 of 2 experiments each)

The two highest doses showing an approximately equivalent potency to epichlorohydrin. Methylnitrosourea had the strongest effect.

Test condition: TEST ORGANISMS:
- Age: ca. 8 weeks
- No. of animals per dose: 10

ADMINISTRATION:
- Vehicle: acetate buffered (pH 6) saline
- Control groups and treatment: solvent only
- MX group: treated with structural analogue to MCA, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H))furanone (MX) at doses of 0, 0.28, 0.37 or 0.46 mmol/kg
- Comparison group: treated with either epichlorohydrin (2.1 mmol/kg bw) or methylnitrosourea (0.4 mmol/kg bw), both known rodent gastrointestinal tract carcinogens

EXAMINATIONS:
- Animals were sacrificed 24 hours after treatment. Tissues from forestomach (200 intact epithelial cells), duodenum and proximal colon (10 complete crypts; ca. 700 - 1000 cells) were assayed for total nuclear anomalies including micronuclei.
- Slides stained by Feulgen method and counter-stained with fast green for histopathological quantification of nuclear anomalies.
- Scoring:
(i) Mincronucleus: same internal structure, shape, and staining intensity as normal nucleus but 1/3 to 1/4 smaller in diameter and clearly disengaged from other nuclear fractions.
(ii) Pyknotic nucleus: no discernable internal structure, darkly stained, usually smaller than a normal nucleus and frequently engulfed in a vacuole.
(iii) Karyorrhectic nucleus: fragmented small nuclear bodies usually arranged in clusters, darkly stained with no internal structure, and sometimes vacuolated.

STATISTICAL ANALYSIS:
log rank test with regard to the number of nuclear anomalies per animal at each tissue site; pairwise comparisons to control group (one-tailed tests) and trend analysis (Fisher's Exact Test)

Conclusion: The authors concluded that mucochloric acid appears to have a "marginal activity" only in the duodenum, and its potency is smaller than that of MX. Overall the relatively weak response was considered not commensurate with the extreme bacterial mutagenicity, which is considered to be indicative of an effective
detoxification mechanism in mammalian cells.

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP. Focus of the study on total nuclear anomalies which can not
directly be attributed to genotoxicity. Only the parameter %animals with micronuclei can be evaluated in the light of in vivo genotoxicity

Flag: Critical study for SIDS endpoint

18-JUN-2003 (92)

Type: other: Cytogenetic monitoring, biomarker for chromosome damage
Species: human **Sex:**
Exposure period: 11.9 years (1 - 17 years)
Doses: concentration at workplace: no measurements available
Result: negative

Year: 1989
GLP: no
Test substance: other TS: no data, but presumably exposure to technical MCA of different degress of purity

Remark: Chromosome analyses were performed in 30 workers handling mucochloric acid. Exposure period was 11.9 years (median, range 1-17 years). Measurements of concentrations at the workplace were not available. Comparison of the structural aberrations (3000 metaphases analyzed) showed no significant difference between exposure (3.0% incl. and 1.4% excl. gaps) and control group (2.9% incl. and 1.2% excl. gaps). (see chap. 5.10).
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Documentation limited.
Flag: Critical study for SIDS endpoint

05-JUN-2003 (93)

Type: other: Slot blot assay to characterize aldehydic DNA lesions and quantitative 8-oxoguanine formation for details see free text
Species: rat **Sex:** male
Strain: Fischer 344
Route of admin.: gavage
Exposure period: single administration
Doses: 0, 10, 30, 100 or 300 mg/kg bw
Result: negative

Method: other: see freetext
Year: 2003
GLP: no data
Test substance: other TS: mucochloric acid not specified

Result: ALDEHYDIC DNA LESIONS (including AP sites):
- no significant differences between control and exposed rat liver

QUANTITY OF 8-OXOGUANIN:
- no significant differences between control and exposed rat liver
Test condition: ANIMAL STUDY:
- 5 dose groups of 6 male animals each
- Strain: Fisher F334 rats

- Dose levels: 0; 10; 30; 100; 300 mg/kg bw

a) Slot blot assay to characterize abasic DNA sites (AP sites = apurinic/apyrimidinic sites)

b) Slot blot assay to quantitate 8-oxoguanine by HPLC-ECD
Reliability: (4) not assignable
Abstract
07-JUL-2003 (94)

5.7 Carcinogenicity

Species: mouse **Sex:** male/female
Strain: other: hybrids B6C3F1 (C57BL/6xAKR)F1 and B6AKF1 (C57BL/6xC3H/Anf)F1
Route of administration: oral feed
Exposure period: 18 months
Frequency of treatment: continuously
Post exposure period: none
Doses: 56 ppm corresponding to ca. 7 mg/kg bw per day (see freetext for further details)
Control Group: other: (i) untreated animals; (ii) vehicle control; (iii) 7 positive control groups

GLP: no
Test substance: other TS: Mucochloric acid, not specified ("commercial source"; no further purification)

Result: MORTALITY: no significant effects compared to vehicle controls (see also chap. 5.4)
NECROPSY FINDINGS: no significant increase of tumour incidences compared to untreated controls
Test condition: In this study, 120 substances were investigated.
TEST ORGANISMS
- Age: 7 days
- Number of animals: 18 mice of each sex and each strain

ADMINISTRATION
(i) 21.5 mg/kg in 0.5% gelatin bw once daily by stomach tube until age of 4 weeks (dose not readjusted according to body weigth gain), followed by (ii) ad libitum administration of 56 ppm TS, mixed into diet, after weaning until end of exposure period (corresponding to ca. 7 mg/kg bw per day)
Both doses were recorded as maximum tolerated doses by the authors, but no data available.

OBSERVATIONS AND FREQUENCY
Animals were observed daily for any abnormalities.

ORGANS EXAMINED AT NECROPSY:
- Macroscopic: thoracic and abdominal cavities
- Microscopic: major organs and all grossly visible lesions (thyroid gland not examined); following tumour groupings analyzed: hepatomas, pulmonary tumours, lymphomas, and total mice with tumours
OTHER EXAMINATIONS: blood smears examined only in cases showing splenomegaly and lymphadenopathy

STATISTICAL METHODS: (i) chi-square tests to test for differences among the 5 negative control groups; (ii) significance test according to Mantel-Haenszel procedure to test for differences in the relative risks (as compared to the controls)
Reliability: (3) invalid

Methodological deficiences: only one dose; number of animals limited; limited number of organs examined; limited tumour categories analyzed
Flag: Critical study for SIDS endpoint
13-MAY-2004 (64) (65)

Species: rat **Sex:** male
Strain: Fischer 344
Route of administration: drinking water
Exposure period: 6 weeks
Frequency of treatment: continously
Post exposure period: 1 week
Doses: 0.45 or 0.90 mg/ml (corresponding to total dose of 43 and 77 mg/kg bw, respectively)
Result: negative
Control Group: yes, concurrent vehicle

Method: other: Detection of aberrant crypt foci (ACF) and intestinal tumours after initiation with 1,2-dimethylhydrazine and treatment with test substance
GLP: no data
Test substance: other TS: Mucochloric acid, >98% purity

Result: ABERRANT CRYPT FOCI (ACF):
Incidence of rats with (i) ACF; (ii) No. of ACF/colon; (iii) ratio AC/ACF (+/- standard deviation)
- vehicle/Aq. dest.: (i)1/5; (ii)0.2+/-0.4; (iii)0.4+/-0.9
- vehicle/43 mg/kg day TS: (i)0/5; (ii)-; (iii)-
- vehicle/77 mg/kg day TS: (i)1/5; (ii)0.2+/-0.4; (iii)0.2+/-0.4
- DMH/Aq. dest.: (i)5/5; (ii)10.8+/-6.5; (iii)2.9+/-0.7
- DMH/42 mg/kg day TS: (i)5/5; (ii)14.0+/-14.1; (iii)2.7+/-0.6
- DMH/76 mg/kg day TS: (i)5/5; (ii)14.0+/-4.3; (iii)2.6+/-0.4

EVALUATION OF RESULTS:
No statistically significant effect of TS on the induction of ACF by DMH

Test condition: TEST ORGANISMS

- Age: 6 weeks
- Number of animals: 5 per dose group

ADMINISTRATION / EXPOSURE
(1.) Direct induction experiment: administration of TS with drinking water ad libitum; 2 dose groups receiving water with 0.45 or 0.9 mg/ml for 6 weeks

(2.) Co-induction experiment:
(i) initiation: two subcutaneous injections of 10 mg/kg bw 1,2-dimethylhydrazine (DMH) 4 days apart during first week
(ii) treatment: after one week, administration of TS as described above (1.)

- Vehicle: NaCl/EDTA
- Daily doses (total dose) of test substance (TS) applied as calculated from measured daily intake of drinking water:
(i) vehicle/Aq. dest.
(ii) vehicle/43 mg/kg bw day (361 mg) TS
(iii) vehicle/77 mg/kg bw day (626 mg) TS
(iv) DMH/Aq. dest.: 0 mg/kg TS

(v) DMH/42 mg/kg bw day (344 mg) TS
(vi) DMH/76 mg/kg bw day (605 mg) TS

SCORING OF ABERRANT CRYPT FOCI:
recorded in colon and caecum; criteria: increased size, elevated appearance above surrounding mucosa, enlarged pericryptal zone, and more enlarged shape of luminal opening

STATISTICAL METHODS: calculation of means +/- standard deviations; Mann-Whitney rank sum test

Conclusion: Authors of this study concluded that no induction of aberrant crypt foci above background was observed.

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; test system not validated for carcinogenicity testing.

Flag: Critical study for SIDS endpoint

03-JUL-2003 (95) (96)

Species: mouse **Sex:** male
Strain: Balb/c
Route of administration: drinking water
Exposure period: 4 weeks
Frequency of treatment: continuously
Post exposure period: 12 weeks
Doses: 0.18 or 0.35 mg/ml (corresponding to total dose of 27 and 59 mg/kg bw, respectively)
Result: negative
Control Group: yes, concurrent vehicle

Method: other: Detection of aberrant crypt foci (ACF) and intestinal tumours after initiation with 1,2-dimethylhydrazine and treatment with test substance
GLP: no data
Test substance: other TS: Mucochloric acid, >98% purity

Result: ABERRANT CRYPT FOCI (ACF):
Incidence of rats with (i) ACF; (ii) No. of ACF/colon; (iii) ratio AC/ACF (+/- standard deviation)
- vehicle/Aq. dest.: (i)4/12; (ii)0.8+/-1.3; (iii)1.0+/-1.5
- vehicle/27 mg/kg day TS: (i)3/5; (ii)1.6+/-2.1; (iii)2.2+/-2.1
- vehicle/54 mg/kg day TS: (i)3/4; (ii)3.3+/-2.8; (iii)3.7+/-3.4
- DMH/Aq. dest.: (i)3/4; (ii)1.5+/-1.7; (iii)1.9+/-1.5
- DMH/27 mg/kg day TS: (i)4/5; (ii)1.8+/-1.3; (iii)5.0+/-4.4
- DMH/59 mg/kg day TS: (i)3/3; (ii)3.7+/-1.2; (iii)3.0+/-1.8

EVALUATION OF RESULTS:
Significant correlation between dose and AC/ACF ratio; small inducing effect on ACF and effect on growth of ACF (AC/ACF). However, no statistically significant effect of all determined
parameters compared to control; no significant effect on the incidences or of induction of ACF by DMH.

Test condition: TEST ORGANISMS
- Age: 6 weeks
- Number of animals: 5 per dose group

ADMINISTRATION / EXPOSURE
(1.) Direct induction experiment: administration of TS with drinking water ad libitum; 2 dose groups receiving water with 0.18 or 0.35 mg/ml for 4 weeks

(2.) Co-induction experiment:
(i) initiation: two subcutaneous injections of 10 mg/kg bw 1,2-dimethylhydrazine (DMH) 5 days apart
(ii) treatment: administration of TS as described above (1.)

- Vehicle: NaCl/EDTA
- Daily doses (total dose) of test substance (TS) applied as calculated from measured daily intake of drinking water:

(i) vehicle/Aq. dest.
(ii) vehicle/27 mg/kg bw day (361 mg) TS
(iii) vehicle/54 mg/kg bw day (626 mg) TS
(iv) DMH/Aq. dest.: 0 mg/kg TS
(v) DMH/27 mg/kg bw day (344 mg) TS
(vi) DMH/59 mg/kg bw day (605 mg) TS

SCORING OF ABERRANT CRYPT FOCI:
recorded in colon and caecum; criteria: increased size, elevated appearance above surrounding mucosa, enlarged pericryptal zone, and more enlarged shape of luminal opening

STATISTICAL METHODS: calculation of means +/- standard deviations; Mann-Whitney rank sum test

Conclusion: Authors of this study concluded that no induction of aberrant crypt foci above background was observed.

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; test system not validated for carcinogenicity testing.

Flag: Critical study for SIDS endpoint

03-JUL-2003 (95) (96)

Species: rat **Sex:** male
Strain: Fischer 344
Route of administration: other: intrarectal instillation
Exposure period: 5 weeks
Frequency of treatment: 3 times per week
Post exposure period: 4 weeks
Doses: 10 or 20 mg/kg bw (total dose: 160 or 320 mg/kg bw)
Control Group: yes, concurrent vehicle

Method: other: Detection of aberrant crypt foci and intestinal tumours after initiation with 1,2-dimethylhydrazine and treatment with MCA
GLP: no data
Test substance: other TS: Mucochloric acid, >98% purity

Result: ABERRANT CRYPT FOCI (ACF):
Incidence of rats with (i) ACF; (ii) No. of ACF/colon; (iii) ratio AC/ACF (+/- standard deviation)
- vehicle/Aq. dest.: (i)2/5; (ii)0.4+/-0.4; (iii)3.2+/-6.1
- vehicle/160 mg/kg bw TS: (i)0/5; (ii)-; (iii)-
- vehicle/320 mg/kg bw TS: (i)1/5; (ii)0.2+/-0.4;

(iii)0.8+/-1.8
- DMH/Aq. dest.: (i)5/5; (ii)6.2+/-3.6; (iii)2.4+/-0.6
- DMH/160 mg/kg bw day TS: (i)5/5; (ii)3.6+/-1.1; (iii)2.9+/-0.4
- DMH/320 mg/kg bw day TS: (i)5/5; (ii)2.8+/-1.6; (iii)3.9+/-1.0 *)

*) siginificantly (P<0.05) different from control

EVALUATION OF RESULTS:
Small effect on growth of preformed aberrant crypt foci indicated by slight, but statistically significant (P<0.05) increase in crypt multiplicity parameter aberrant crypts/aberrant crypt foci observed in the highest dose group. No other significant effects.

Test condition: Rats received two s.c. injection of 1,2-dimethylhydrazine (10 mg/kg bm) or the vehicle NaCl/EDTA four days apart during the first week. After one week MCA (10 or 20 mg/kg bw) or water was given by intrarectal intubation of 0.3 ml three times per week for about 5 weeks, totally 16 times, giving total doses of 160 or 320 mg/kg bw or 1.5 or 2.9 mg per rat. Rats were terminated four weeks after the last intubation and aberrant crypt foci and intestinal tumours.

Conclusion: Authors of this study did not exclude the possibility that "the apparent effect od MCA on growth of aberrant crypt foci is due to chance, cause by large variation in these experiments."

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; test system not validated for carcinogenicity testing.
Study not considered as key study because administration route not relevant way of exposure.

Flag: Critical study for SIDS endpoint

18-JUL-2002 (96)

Species: mouse **Sex:** male
Strain: Balb/c
Route of administration: other: intrarectal instillation
Exposure period: 4 weeks
Frequency of treatment: three times per week
Post exposure period: 12 weeks
Doses: 5 or 10 mg/kg bw (total dose: 55 or 110 mg/kg bw)
Result: ambiguous
Control Group: yes, concurrent vehicle

Method: other: Detection of aberrant crypt foci and intestinal tumours after initiation with 1,2-dimethylhydrazine and treatment with MCA
GLP: no data
Test substance: other TS: Mucochloric acid, >98% purity

Result: ABERRANT CRYPT FOCI (ACF):
Incidence of rats with (i) ACF; (ii) No. of ACF/colon; (iii) ratio AC/ACF (+/- standard deviation)
- untreated controls: (i)4/12; (ii)0.8+/-1.3; (iii)1.0+/-1.5
- vehicle/55 mg/kg bw TS: (i)4/6; (ii)2.5+/-2.7; (iii)2.6+/-2.4
- vehicle/110 mg/kg bw TS: (i)4/4*); (ii)3.3+/-2.6;

(iii)3.1+/-1.8*)
- DMH/Aq. dest.: (i)4/5; (ii)2.8+/-1.7; (iii)4.5+/-3.0
- DMH/55 mg/kg bw TS: (i)5/5; (ii)2.0+/-1.4; (iii)6.1+/-3.5
- DMH/110 mg/kg bw TS: (i)3/3; (ii)5.0+/-1.0; (iii)4.7+/-2.2

*) siginificantly (P<0.05) different from control

EVALUATION OF RESULTS:
MCA had a weak and dose-dependent effect on the induction of aberrant crypt foci. Also the growth of these foci seemed to be enhanced as measured by the parameter aberrant crypts/aberrant crypt foci. No significant effect observed when MCA was given after 1,2-dimethylhydrazine.

Test condition: Mice received two s.c. injection of 1,2-dimethylhydrazine (10 mg/kg bm) or the vehicle NaCl/EDTA five days apart. After that MCA (5 or 10 mg/kg bw) or water was given by intrarectal intubation three times per week, totally 11 times, giving total doses of 55 or 110 mg/kg bw or 1.5 or 2.9 mg per mouse. The mice were terminated 17 weeks after start of the experiment and aberrant crypt foci and intestinal tumours.

Conclusion: Authors of this study did not exclude the possibility that "the apparent effect od MCA on growth of aberrant crypt foci is due to chance, cause by large variation in these experiments."

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; well documented; acceptable for assessment.
Restrictions: Study not conducted in accordance with standard test guidelines or GLP; test system not validated for carcinogenicity testing.
Study not considered as key study because administration route not relevant way of exposure.

Flag: Critical study for SIDS endpoint

03-JUL-2003 (95) (96)

Species: mouse **Sex:** male/female
Strain: other: (C57BL/6xAKR)F1 (C57BL/6xC3H/Anf)F1
Route of administration: s.c.
Exposure period: single treatment
Post exposure period: 18 months
Doses: 21.5 mg/kg in DMSO (0.05 ml)
Control Group: other: untreated animals, vehicle control, 7 positive controls

GLP: no

Result: In this study, 120 substances were investigated. MCA was tested in 18 male and 18 female animals of each of 2 strains of mice. There was no indication of an increased tumor incidence compared to negative controls.

Reliability: (3) invalid
Methodological deficiences: only one dose; number of animals limited; limited number of organs examined; limited tumour categories analyzed

05-APR-2002 (65)

Species: other: two-stage cell **Sex:**

transformation assay in vitro
Exposure period: see freetext
Frequency of treatment: see freetext
Post exposure period: see freetext
Doses: see freetext
Result: positive
Control Group: other: see freetext

Method: other: non validated method according to Laakson et al. 2001, Arch Toxicol 75: 613-617
Year: 2003
GLP: no
Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Remark: Study not flagged critical for SIDS endpoint due to use of non-validated test method and release after SIAM descission. Study results do not alter allover evaluation in the SIAP.
Result: Significancies:
a: alpha = 0.05 significantly different from the control group no treatment
b: alpha = 0.05 significantly different from the acetone only group (solvent for MC)
c: alpha = 0.05 significantly different from the MC only group
d: alpha = 0.05 significantly different form the corresponding MCA only group

Colony forming efficiency [%]:
- no treatment: 100
- TPA only:
- Acetone only: 106
- Acetone + TPA:
- MC only: 112.8
- MC + TPA
- MC + MCA [1.0 µg/ml]:
- MC + MCA [2.0 µg/ml]:
- MC + MCA [4.0 µg/ml]:
- MCA only [5 µg/ml]: 96.4
- MCA [5 µg/ml] + TPA:
- MCA only [10 µg/ml]: 96.6
- MCA [10 µg/ml] + TPA
- MCA only [15 µg/ml]: 81.8
- MCA [15 µg/ml] + TPA

Transformation [Total no. foci/no. dishes examined]:
- no treatment: 21/12
- TPA only: 34/12
- Acetone only: 17/12
- Acetone + TPA: 35/12
- MC only: 97/12
- MC + TPA: 131/14
- MC + MCA [1.0 µg/ml]: 118/10
- MC + MCA [2.0 µg/ml]: 160/12
- MC + MCA [4.0 µg/ml]: 117/12
- MCA only [5 µg/ml]: 25/13
- MCA [5 µg/ml] + TPA: 33/11
- MCA only [10 µg/ml]: 18/12
- MCA [10 µg/ml] + TPA: 29/12
- MCA only [15 µg/ml]: 22/12
- MCA [15 µg/ml] + TPA: 58/12

Transformation [Foci/dish]:
- no treatment: 1.8
- TPA only: 2.8a
- Acetone only: 1.4
- Acetone + TPA: 2.9
- MC only: 8.1b
- MC + TPA: 9.4
- MC + MCA [1.0 µg/ml]: 11.8c
- MC + MCA [2.0 µg/ml]: 13.3c
- MC + MCA [4.0 µg/ml]: 9.8
- MCA only [5 µg/ml]: 1.9
- MCA [5 µg/ml] + TPA: 3.0
- MCA only [10 µg/ml]: 1.5
- MCA [10 µg/ml] + TPA: 2.4
- MCA only [15 µg/ml]: 1.8
- MCA [15 µg/ml] + TPA: 4.8d

Transformation [Type II/dish]:
- no treatment: 0.9
- TPA only: 1.0
- Acetone only: 0.8
- Acetone + TPA: 1.5
- MC only: 3.7b
- MC + TPA: 3.2
- MC + MCA [1.0 µg/ml]: 4.5
- MC + MCA [2.0 µg/ml]: 5.5c
- MC + MCA [4.0 µg/ml]: 3.9
- MCA only [5 µg/ml]: 0.5
- MCA [5 µg/ml] + TPA: 1.5
- MCA only [10 µg/ml]: 0.6
- MCA [10 µg/ml] + TPA: 0.8
- MCA only [15 µg/ml]: 0.8
- MCA [15 µg/ml] + TPA: 2.3d

Transformation [Type III/dish]:
- no treatment: 0.1
- TPA only: 0.7
- Acetone only: 0.1
- Acetone + TPA: 0.8
- MC only: 1.7b
- MC + TPA: 3.1
- MC + MCA [1.0 µg/ml]: 3.8c
- MC + MCA [2.0 µg/ml]: 5.0c
- MC + MCA [4.0 µg/ml]: 3.7c
- MCA only [5 µg/ml]: 0.6
- MCA [5 µg/ml] + TPA: 0.9
- MCA only [10 µg/ml]: 0.8
- MCA [10 µg/ml] + TPA: 0.5
- MCA only [15 µg/ml]: 0.6
- MCA [15 µg/ml] + TPA: 1.0

Initiation phase:
- no induction of foci development by MCA on its own
- TPA statistically significant increased the foci per dish and type II foci numbers in cells treated with 15 µg/ml MCA during initiation phase

Promotion phase
- MCA at 1.0 and 2.0 µg/ml increased statistically significant number of foci per dish in MC initiated cells
- MCA at 2.0µg/ml increased statistically significant number

of type II foci per dish
- MCA at 1.0, 2.0 4.0 µg/ml increased number of type III foci per dish, however number of type II foci per dish and number of foci per dish not statistically significant increased at highest concentration 4.0 µg/ml (somewhat inverse dose response)

Test condition: Test system: C3H 10T1/2

Test concentration: during initation stage: 5, 10 and 15 µg/ml; during promotion stage: 1,2 and 4 µg/ml

Cytotoxic concentration: not given

Without metabolic activation

Chemicals and Controls:
- mucochloric acid (MCA)
- 3-methylcholanthrene (MC) from ICN Biomedicals Inc (Aurora, OH, USA) as positive control for initiation
- 12-O-tetra-decanouylphorbol-13-acetat (TPA) from ICN Biomedicals Inc (Aurora, OH, USA) as positive control for promotion
- acetone from SIGMA (St. Louis, MO, USA) as solvent control for MC during initiation phase
- Dulbecco's modified eagle medium (DMEM) from Gibco (Stockholm, Sweden) as solvent control during promotion phase
- dimethylsulfoxyde (DMSO) from Merck (Darmstadt, Germany)
- for stock solutions MC was dissolved in acetone, TPA in DMSO and MCA in DMEM from Gibco (Stockholm, Sweden) without supplements
- dilution of stock solution with DMEM

Cells, media and culture conditions:
- contact sensitive C3H 10T1/2 mouse embryonic fibroblasts (cell line ATCC CCL-226)
- DMEM medium containing 10% FCS, 100 IU/ml penicillin, 100 µg/ml streptomycin
- at 37°C in humidified incubator, 5% CO2
- cell culture flasks and 60-mm dishes
- storage in ampoules frozen in liquid nitrogen
- preparation of stock solutions between passages 2 and 4 after having been supplied to the laboratory
- cell detachment with 0.05% trypsin and 0.02% EDTA in PBS

Cytotoxicity assay:
- 3 different experiments (except for MC and acetone, where only 1 experiment performed); 5 dishes per experiment
- Cells harvested from logarithmically growing stock solution were plated on day 0 on 60 mm-dishes (200 cells/dish)
- cells grown in the presence of MC or MCA for 3 days
- fixation of colonies 7-9 days after seeding, staining with GIEMSA solution
- counting of colonies at 7-10 days of culture
- criteria: maximum decrease in colony-forming efficiency of 30 to 55%

Transformation assay:
- 3 independent experiments per exposure condition
- MC uses as positive control initiator and TPA as positiv control promotor; concentration MC: 5 µg/ml, TPA: 0.3 µg/ml

- concentration selection of mucochloric acid (MCA) based on results of cytotoxicity assay (see above)
- Cells harvested from logarithmically growing stock solution were plated on day 0 on 60 mm-dishes (10^3 cells/dish)
- after 24 h incubation with initiating agent (MC, MCA or vehicle control) for 72 h
- medium renewal and further growing of cells in fresh medium for 7 days
- incubation during tumor-promoting phase with either TPA or MCA or DMEM as a solvent control for 14 days
- subsequent growing of cells for another 2 weeks in medium without chemical agents
- throughout the study change of medium weekly
- fixation with ethanol; staining with Giemsa solution; rinsing 3times with water; air drying
- Counting of transformed foci: as defined by Reznikoff et al. 1973, Cancer Res 33: 3239-3249; using an inverted microscope

Definition of foci:
Typ I: some tightly packed cells
Typ II: focus showing more massive build-up of cells into virtually opaque multilayers, with no pronounced criss-cross pattern
Typ III: focus of highly polar, multilayered criss-cross arrays of densely stained cells

Statistical analysis:
- Kruskal-Wallis test (Conover 1999)
- differences at alpha = 0.05 were considered statistically significant

Conclusion: MCA alone did not cause initiation but promoted MC-induced foci development

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments; no guideline study; in vitro results; method not validated, transformation assay with this cell line (C3H 10T1/2) not accepted for validation by ECVAM

30-JUL-2004

5.8.1 Toxicity to Fertility

Type: Fertility

Remark: There are no fertility studies available.
MCA is a corrosive substance used mainly in closed systems as a chemical intermediate. Transport of the isolated material is controlled and is limited to a very small number of sites. Exposure is controlled in occupational settings and is negligible for consumers.
Because of its corrosive properties, and the limited exposure potential, animal tests with MCA for its effects on fertility were not performed.

Flag: Critical study for SIDS endpoint

05-AUG-2003

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: day 6 to 19 post coitum
Frequency of treatment: once daily
Duration of test: 20 days
Doses: 5, 30 or 60 mg/kg bw/day
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: = 5 mg/kg bw
NOAEL Teratogenicity: = 60 mg/kg bw

Method: OECD Guide-line 414 "Teratogenicity"
Year: 2001
GLP: yes
Test substance: other TS: Mucochloric acid, techn. pure 99.3% (2x recrystallized); white, solid/crystalline

Method: METHOD FOLLOWED: OECD guideline No. 414 draft of June 2000 and final version of 22 January 2001; US EPA OPPTS 870.3700, August 1998; EC Commission Directive 87/302/EEC Nov. 18, 1987

DEVIATIONS FROM GUIDELINE: none reported

Result: CHEMICAL ANALYSIS OF THE DOSAGE FORMS - ANALYTICAL CONCENTRATIONS:
- Confirmed that dispersions of TS were homogeneous
- Measured analytical concentration of TS in dosage preparations (3 samples each):
(i) 1st day: 0, 88.4, 94.3 or 100% of nominal concentrations of 0, 1.67, 10 or 20 mg/ml, respectively;
(ii) Last day: 0, 95.4, 97.0 or 93.6% of nominal concentrations of 0, 1.67, 10 or 20 mg/ml, respectively

MATERNAL TOXIC EFFECTS BY DOSE LEVEL:
- Test groups: (1) 0 mg/kg bw/day; (2) 5 mg/kg bw/day; (3) 30 mg/kg bw/day; (4) 60 mg/kg bw/day
- Number pregnant per test group: (1) 23; (2) 20; (3) 19; (4) 18 (+ 1 pregnant found dead on day 14 p.c.) (according to OECD and US EPA guidelines ca. 20 but not fewer than 16 females with implantation sited required)

CLINICAL EXAMINATIONS:
- Mortality: no deaths in all test groups except for 1 female found dead on day 14 p.c. in group 4 (60 mg/kg bw/day); this is considered as incidential, not substance-related death, since no abnormalities in food consumption or body weight were recorded before the death and no findings were obtained on necropsy.

- Clinical symptoms: groups 1, 2 and 3: no remarkable signs; group 4: ptyalism in 24/25 females (day 13-17 p.c. until

termination) indicating poor GI tolerance due to corrosive properties of test substance; loud breathing in 11/25 probably due to compensatory mechanism.

- Food consumption: group 1 and 2 similar; groups 3 and 4: significantly reduced (-8%) during first 3 days, not statistically significantly reduced (-4%) on days 6-20.

- Body weight: group 1 and 2 similar; groups 3 and 4: clearly reduced during first 3 days(-23%; p<0.05 at 30 mg/kg bw/day; -14%, not statistically significant at 60 mg/kgbw/day).

- Corrected body weight gain: group 1 and 2 similar; groups 3 and 4: reduced (-13%; not statistically significant at 30 mg/kg bw/day; -17%, p<0.05 at 60 mg/kgbw/day), considered as treatment-related.

EXAMINATION OF DAMS AT TERMINATION
- Uterus weight: slightly increased in all treated groups due to higher litter sizes, which was considered to be by chance and, thus, of no biological significance.

- Necropsy findings: group 1 and 2: no macroscopic findings; groups 3 and 4: no relevant findings except for whitish foci in the stomach of 4/25 and 15/25, respectively.

- Reproduction data: conception rate 92% (group 1 = control); 80% (group 2), 76% (group 3), 72% (group 4); no substance-related and/or biologically relevant differences between all test groups regarding mean number of copora lutea and implantation sites or in the values calculated for pre and post-implantation losses, number of resorptions and viable fetuses. Slightly higher number of fetuses per litter in treated groups, which was considered to be by chance and, thus, of no biological significance

EXAMINATION OF FETUSES:
- Sex ratio: similar in all groups 1-4 and close to normal value of 50%.

- Weights of fetuses: similar in all groups 1-4

- External malformations: groups 1-3: none at all; group 4: 1/221 fetuses with thread-like tail, considered as spontaneous occurrence.

- External variations: none in any group.

- Soft tissue malformations: none in any group.

- Soft tissue variations: confined to dilatation of renal pelvis and/or ureters.

Incidence of dilatation of renal pelvis similar in groups 1-3, but slightly higher in group 4, although not statistically significant and within range of historical control data.
Incidence of dilatation of ureters in group 4 also not statistically significant, but slightly above range of historical control data. Dilatation unilateral in 8/10 cases and no dose-related trend.

- Skeletal malformations: none in any group.

- Skeletal variations: similar in all groups with regard to nature and incidence. Two exceptions: (i) higher incidence of incomplete ossification of 5th sternebra in group 3; (ii) higher incidence of incomplete ossification of 1st to 4th

sternebrae in group 2. Both effects within range of historical control data and not occurring in high-dose group; thus, considered as spontaneous occurrence.

- Fetal skeletal cartilage examination: cartilage generally observed in a similar manner in all groups, i.e. at skeletal structures incompletely ossified or unossified, confirming that the variations corresponded to fluctuations in ossification degree and not to permanent alterations.

EVALUATION:
NOAEL for maternal toxicity: 5 mg/kg bw/day based on reduced food consumption and body weight gain at 30 and 60 mg/kg bw/day; whitish foci in these groups and ptyalism in highest dose group being considered as local effects due to corrosive properties and hence, poor GI tolerance of test substance.

NOAEL for prenatal developmental toxicity: 60 mg/kg bw/day

Test condition: TEST ORGANISMS
- Age: 11 weeks
- Weight at study initiation: on average 243 g (range 189-302 g)
- Number of animals: 100 (25 per group)

ADMINISTRATION / EXPOSURE
- Vehicle: olive oil
- Dosage form preparation: test substance ground to fine powder, suspended in vehicle and homogenized; freshly prepared daily and administered to animals within 2 hours; stability in olive oil is guarenteed for 96 hours
- Concentration in vehicle: 1.67, 10 or 20 mg/ml
- Total volume applied: 3 ml/kg bw/day
- Analysis of test substance preparations for concentration (HPLC) and homogeneity on first and last day of treatment

EXAMINATIONS OF DAMS AND FETUSES: according to guideline used

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
19-JUL-2002 (63)

5.8.3 Toxicity to Reproduction, Other Studies

-

5.9 Specific Investigations

-

5.10 Exposure Experience

Type of experience: Health records from industry

Remark: From 1955-1971 74 cases of occupational dermatoses caused by
mucochloric acid and its by-products were registered in a chemical plant. No further details of the cases were given

(see also chap. 5.3).
Reliability: (3) invalid
No details reported
02-JUN-2003 (62)

Type of experience: other: Biomarker for chromosome damage

Remark: Chromosome analyses were performed in 30 workers handling mucochloric acid. Exposure period was 11.9 years (median, range 1-17 years). Measurements of concentrations at the workplace were not availbale. Comparison of the structural aberrations (3000 metaphases analyzed) showed no significant
difference between exposure (3.0% incl. and 1.4% excl. gaps)
and control group (2.9% incl. and 1.2% excl. gaps).
Test substance: No exposure data, but presumably exposure to technical MCA of different degress of purity
Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards; acceptable for assessment.
Restrictions: Documentation limited to the above.
Flag: Critical study for SIDS endpoint
22-JUL-2002 (93)

Type of experience: other: Accidental occupational exposure

Remark: Contamination with mucochloric acid and 1-phenyl-4,5-dichloropyridazone-6 in 7 workers in the pyramine production resulted in a second degree burn appearing after a latency period of several hours. Six to ten days later digestive disorders and slight liver enlargement in patient with more extensive local injuries enmerged. Increased SGPT, LDH, and proteins were found in all patients.
Conclusion: It should be noted that the observed effects cannot be related to MCA due to the multiple exposure situation.
Reliability: (4) not assignable
Only abstract available
18-JUL-2002 (97)

5.11 Additional Remarks

Type: other: Bacteriostatic effects

Remark: The test substance was bacteriostatic to E. coli and Staphylococcus aureus, but not virostatic to specific phagi.
Test substance: Mucochloric acid, pure, neutralisized

03-JUL-2003 (98)

Type: other: Bacteriostatic effects

Remark: The test substance was bacteriostatic to E. coli and Staphylococcus aureus, but not virostatic to specific phagi.
Test substance: as prescribed by 1.1 - 1.4 (mucochloric acid, pure)

17-JUN-2003 (98)

Type: other: Mechanism-based structure-activity relationship analysis

Result: MCA:
M = moderate = likely to be a moderately active multispecdies/targe carcinogen at relatively high doses or active single species/target carcinogen at low doses

Test condition: Categorization by semiquantitative ranking scale; based on expert judgment relative to known carcinogens:
- H = high = highly likely to be a potent multispecies, multitarget carcinogen even at low doses
- HM = high-moderate = highly likely to be an active multispecies/target carcinogen at moderate doses
- M = moderate = likely to be a moderately active multispecdies/targe carcinogen at relatively high doses or active single species/target carcinogen at low doses
- LM = low-moderate = likely to be weakly carcinogenic or carcinogenic toward a single species/target at relatively high doses
- Mar = likely to have marginal carcinogenic activity or may be weakly carcinogenic at doses at or exceeding maximum tolerated doses
- L = unlikely to be carcinogenic

Reliability: (2) valid with restrictions
Structure activity relationship analysis by expert judgement

Flag: Critical study for SIDS endpoint

17-JUN-2003 (99)

Type: other: QSAR of mutagenicity of chlorohydroxyfuranones

Result: Mutagenicity is mainly a manifestation of electron-accapting ability

Test condition: COMPUTATIONAL METHODS:
- AM1 calculations (method: Dewar MJS, Zoebisch EG, Healy EF, Stewart JJP (1985) J Am Chem Soc 107: 3902-3909) with AMPAC program package (QCPE No 506 version 2.1) on a VAX 300 computer
- all geometrical variables completely optimizeid for each compound
- Electron affinity calculation: Difference in total energy between neutral molecul and corresponding anion radical
- Frontier electron density for nucleophilic reaction: Approximative method of Sayama et al (1990) (Sayama M, Mori M, Shinoda H,, Kozuka H (1990) Mutation Res 243: 47-52)
- Calulation of deprotonation enthlapies (method: Dewar MJS, Dieter KM (1986) J Am Chem Soc: 108: 8075-8086)
- Average molecular polarizability (method: Miller KJ, Savchik JA (1979) J Am Chem Soc 101: 7206-7213
- Factor analysis including principle component analysis without rotation and with VARIMAX rotation using SPSS software package

ESTIMATION OF HYDROPHOBICITY:
- octanol-water partion coefficient log P according to the method of Klopman et al. 1985 (Klopman G, Namboodiri K, Schochet J (1985) J Comput Chem 6: 28-38)
- C LOG P values (method: Leo A (1988) Medicinical Chemistry Project, Pomona College Claremont CA)

MUTAGENICITY DATA:
from literature:
- Ishiguro Y, LaLonde RT, Dence CW, Santodonato J (1987) Environ Toxicol Chem 6: 935-946
- Ishiguro Y, Santodonatro J, Neal MW (1988) Environ Mol Mutagen 11: 225-234
- LaLonde RT, Perakyla H, Cook GP, Dence CW (1990) Environ Toxicol Chem 9: 687-691
- LaLonde RT, Cook GP, Perakyla H, Bu L (1991a) Chem Res Toxicol 4: 540-545
- LaLonde RT, Cook GP, Perakyla H, Dence CW (1991b) Chem Res Toxicol 4: 35-40
- LaLonde RT, Cook GP, Perakyla H, Dence CW, Babish JG (1990c) Environ Mol Mutagen

Reliability: (2) valid with restrictions
Accepted SAR method

Flag: Critical study for SIDS endpoint

17-JUN-2003 (100) (101)

Type: other: QSAR of mutagenicity of chlorohydroxyfuranones

Result: Mechanism for the mutagenic activity of halogenated furanones in Salmonella typhimurium TA 100 tester strain:
- one-electron reduction as a key step; thermodynamic mechanism and not site-specific binding or adduct formation

Reliability: (2) valid with restrictions
Accepted SAR method

Flag: Critical study for SIDS endpoint

17-JUN-2003 (102)

Type: other: QSAR of mutagenicity of chlorohydroxyfuranones

Result: - strong negative correlations of LUMO and radical anion stability against log mutagenicities (all 10 compounds)
- no correlation of HOMO energies and mutagenicity

Test condition: Chemicals:
MX and 9 related compounds including MCA

Computional method:
- semiempirical molecular orbital calculations with MOPAC versions 6.0 running on a VAX 8610
- Usage of MNDO-PM3 Hamiltonian
- Optimization of bond length, bond angles and dihedral angles using default HFGS method
- radical anion calculation with and without unrestricted Hartres-Fock (UHF/MNDO) method
- Symmetry option: bond length of identical substituents on C-6 of MX and its derivatives and for hydrogen atoms on C-5 of the reduced compounds
- Keyword used: PRECISE (Increase in default criteria for termination by factor 100)

Mutagenicity assay:
- Standard plate Ames test without metabolic activation
- Tester strain: Salmonella typhimurium TA 100
- Solvent: DMSO
- 3 plates per dose level
- Determination of mutagenicity as revertants/µg from the

linear portion of the dose-response plot
- Spontaneous mutants in solvent control were taken as zero-dose point
- Controls:
  - Solvent (DMSO)
  - Crystal Violet
  - Ampicillin
  - Sodium azide

Statistical treatment of data:
- Values for computed and experimental properties of 10 compounds were plotted against the log of each of 58 mutagenicity values

- Determination of slopes by least-square method

Reliability: (2) valid with restrictions
Scientifically accepted SAR method

Flag: Critical study for SIDS endpoint

18-JUN-2003 (103)

Type: other: QSAR of mutagenicity of chlorohydroxyfuranones

Result: MUTAGENICITY:
Net revertants/µmol: MCA: 4,021; 9,276; 7,243
3,4-dichloro-2(5H)-furanon: 100; 173; 170

- MCA more mutagenic than 3,4-dichloro-2(5H)-furanon indicating that hydroxyl group substituted at 5 position has marked influence on mutagenicity

Test condition: Compounds:
-2,3-dichloro-5-methoxy-2(5H)-furanone
-2,3-dichloro-4,4-dimethoxy-2-butenoate
-3,4-dichloro-2(5H)-furanone

Spectrometric measurements
IR spectra:
- Perkin-Elmer 1310 spectrometer
GC-MS (Gas chromatographic electron impacte (EI) mass specrometry)
- Finnigan 4021 spectrometer at 70 eV
- in conjunction with a 30-meter SPB-5 capillary column - operated initially at 50 °C for 2 min; therafter linearly increased to 300 °C at rate of 10 °C/min
- flow of helium carrier gas: 1 ml/min
1 H NMR and 13C NMR
- Varian EM 360 resp. Varian XL 100 spectrometer
- solvent: deuterated chloroform
- internal standard tetramethylsilane (TMS)
UV spectra:
- Kontron UVIKON 860 spectrometer
- immediately after preparation as well as 4 and 24 h after storage at 37 °C
- Solvent: 10 ml DMSO
- Addition of 9 ml 0.1 M citric acid
- Dilution to 100 ml with pH 7 buffer (citric acid-NaHPO4) solution
- Experiment repition at pH 5

Mutagenesis assay

- Ames test according to Maron and Ames (1983) Mutat Res 113: 173-215
- Tester strain: (his-) S. typhimurium TA 100
- Controls:
Solvent: DMSO
Positive: MMS
- Standard plate assay without metabolic activation

- three plates per dose level
- 3 assays per compound for MCA and
3,4-dichloro-2(5H)-furanone always tested in parallel
- dose ranges:
MCA: assay A: 0; 0.5; 1; 2; 4 µg/plate; assay B: 0; 0.2; 0.4; 0.8; 1 µg/plate and assay C: 0.2; 0.4; 0.6; 0.8; 1.0 µg/plate; 4 µg/plate toxic dose level
3,4-dichloro-2(5H)-furanon: assay A: 0; 5; 10; 20 µg/plate; assay B: 0; 10; 20; 40 µg/plate; assay C: 0; 5, 10; 15; 20 µg/plate; 40 µg/plate toxic dose level
- Results as revertants/µg/plate obtained form the linear portion of the dose -rsponse curves where spontaneous TA 100 mutants in DMSO were taken as zero-dose points

Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Reliability: (2) valid with restrictions
Scientifically accepted SAR method

Flag: Critical study for SIDS endpoint

19-JUN-2003 (104)

Type: other: Reaction with Adenine

Result: Identification of adducts formed in reactions of calf thymus DNA adenine with MCA:
- adduct:
3-(2´-deoxyribofuranosyl)-7-formylimidazo[2,1-i]purine
- yield: 5 adducts/10E6 nucleotides

Test condition: Reactions with calf thymus DNA
- 18.25 mg was reacted with double-stranded calf thymus DNA (3.75 mg) in 1.5 ml of 0.1 M phophate buffer at pH 6.5.
- mixture was stirred and incubated at 37°C for 2 and 4 days
- Monitoring and readjustment of pH during first 12 hours and than twice a day
- DNA recovery by precipitation with ethanol: incubation mixture with 0.2 ml of 5 M NaCl and 3 ml of cold 96% ethanol
- Centrifugation: 10 min at 3000 rpm; removal of supernatant
- Twice repeating of precipitation and centrifugation

Enzymatic hydrolysis of DNA:
- Dissolving of DNA in 3.75 ml of 0.1 M phophate buffer pH 7.4 containing 5 mM MgCl2
- Addition of DNase I (dissolved at 10 mg 0f DNase/ml in 0.9% NaCl) to obtain 0.1 mg of DNase/ml
- Incubation and stirring for 3 h at 37°C
- Addition of Nuclease P1 (dissolved at 0.5 mg/ml in mM ZnCl2) to obtain a final concentration of 20 µg nuclease/ml
- Addition of alkaline phosphatase (87 U/ml in water) and acid phosphatase (20 U/ml in water) to obtain final concentrations of 0.5 and 0.3 U/ml respectively
- Incubation and stirring for 18h at 37°C
- Rotary evaporation of hydrolyzed DNA to near dryeness
- Washing: four times 2.5 ml ethanol/methanol 1:1

- Combination of washes and removal of insoluble particles by centrifugation (20 min, 3000 rpm)
- Rotary evaporation of hydrolyzed DNA to near dryeness
- Addition of 0.1 ml water
- HPLC analysis of 20 µl injectate
- Additionally analysis of insoluble particles dissolved in water

HPLC analysis:
- Kontron liquid chromatographic system: model 322 pump; 440 diode array detector (UV); JASCO FP-920 fluorescence detector; KromaSystem 2000 data handling program
- column: C18 Sperisorb ODS2 analytical column 5 µm (4 x 125 mm); C8 Lichorspher 100 RP-8 column 5 µ (4 x 125 mm); C18 Sperisorb ODS2 analytical column 5 µm (4 x 250 mm)
- Elution: isocratically for 5 min with 5% acetonitrile in water followed by a gradient from 5% to 30% acetonitrile in 25 min at a flow rate of 1 mL/min

Preparative isolation:
- by Column chromatography
- Column: C18 Bondesil bound silica grade 40 µm (2.5 x 10 cm)
- purification on HPLC: Shimadzu LC-9A pumps, variable wavelength Shimadzu SPD 6A UV spectrophotometric detecdtor; Rheodyne injector model 7120 equipped with a 2000 µL loop; injection volume 1ml; column: C18 analytical column (4 x 125 mm)

Spectroscopic and Spectrophotometric methods:
1H NMR spectra:
- JEOL JNM-A500 Fourier transform NMR spectrometer at 500 MHz
- samples dissolved in Me2SO-d6
- internal standard TMS
- determination of shifts and coupling constants in ribosyl units was based on first-order approach
UV-spectra and fluorescence spectra:
- as peaks eluted from the HPLC columns
Mass spectra:
- Fisions ZABSpec-oaTOF instrument
- Ionisation mode: either electron impact or electrospray
- Electron impact: at 70 eV; samples applied through direct inlet probe
- Electrospray: using nitrogen as both nebulizing and bath gas; potential of 8.0 kV applied to the needle;
- temperature of pepperpot counter electrode 90°C;
- sample introduction by loop injection at flow rate of 20 µl/min (H2O/CH3CN/acetic acid: 80/20/1)
- standards: PFK and PEG 200
- resolution of a mass spectrometer: 7000
(1H-NMR-, 13C-NMR-, MS-, UV- spectra)

Test substance: other TS: mucochloric acid; >=98% Source Fluka; see 1.1.1
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

17-JUN-2003 (86)

Type: other: Reaction with Adenosine

Remark: Formation of epsilonoA,A and epsiloncA,A probably by oxidative

properties of MCA

Result: - Two peaks occuring at longer retention times were identified as
3-(beta-D-Ribofuranosyl)7-formyl-8-[9'-(beta-D-ribofuranosyl)-N8-adenonsinyl]imidazo[2,1-i]purine (epsiloncA,A) and
3-(beta-D-Ribofuranosyl)7-oxalo-8-[9'-(beta-D-ribofuranosyl)-N8-adenonsinyl]imidazo[2,1-i]purine (epsilonoA,A)
- The yield of this products increased when the mole ratio of MCA/adenosine was increased from 1:2 to 2:1
- pH influence: at pH 6.0 both beaks were formed in higher amounts compared to pH 7.4
- at 90°C 50% of epsilonoA,A was decarboxylated to epsiloncA,A within 2 hours

Test condition: ANALYSIS:
- Product isolation and sampling with HPLC
- HPLC1: Instrumed containing 2 Shimadzu LC-9A pumps and a Shimadzu SPD-6A UV spectrophotometric detector; detector at 290 nm
- Separation on Spherisorb ODS2 C18 analytical column 5 µm (4 x 125 mm); isocratic elution for 5 min with 5% acetonitrile in 0.01 M potassiumdihydrogen phosphate (pH 4.6) followed by a gradient from 5- to 30% acetonitrile in 25 min at 1 ml/min
- Preparative isolation of products: Column chromatography; Bondesil Column for preparative C18 bonded silica grade 40 µm(2.5 x 10 cm); equilibration with water followed by a batchwise elution with 0%, 5%, 10% and 15% acetonitrile in 100 ml of water
- Fractions further purified by HPLC on a Nucleosil 7 C18 semipraparative column 7 µm (10 x 250 mm); isocratic elution with 8% (fraction of 10% acetonitril) and 13% (fraction of 15% acetonitrile) acetonitrile at a solven flow rate of 4 ml/min
- Fractions containing the pure products were rotary evaporated to dryeness
- 1HNMR and 13CNMR: JEOL JNM-A500 Fourier transform NMR spectrometer at 500 and 125 MHz respectively; samples dissolved in Me2SO-ds; reference standard central peak of the solvent
- Thermospray mass spectrometry system consisting of VG Trio-2 quadrupole mass spectrometer interfaced with a VG thermospray-plasmaspray probe; MS connected to a HPLC system consisting of a Model 2900-0374 solvent delivery system and a Ultrapsphere ODS 5 µm (4.6 x 250 mm) column; column eluted with 0.1 M ammonium acetate : acetonitrile (70:30) at pH 4.6
- UV spectra: Shimadzu UV -160 spectrophotometer

REACTIONS WITH NUCLEOSIDES:

a) Reaction at 37 °C

- 0.119 mol (20 g) MCA was added to 4 l of a 0.5 M phosphate buffer solution, pH 7.4 containing 0.059 mol (15.9 g) adenosine.
- Reaction for 5 days
- Followed by HPLC analysis: Spherisorb ODS2 C18 analytical column 5 µm (4 x 125 mm)
- Reaction mixture filtered and passed through manually packed Bondesil Column for preparative C18 bonded silica grade 40 µm (2.5 x 10 cm)
- Fractions of 10% and 15% acetonitrile were collected and their volumn reduced to about 30 ml by rotary evaporation
- Purification by use of Nucleosil 7 C18 semipraparative

column 7 µm (10 x 250 mm);
- rotary evaporated products subjected to spectrometric studies

b) Reaction at various mole ratios of MCA to adenosine and pH conditions
- Adenosine 10.5 mg (0.039 mmol) was reacted with 3.3 mg (0.019 mmol), 6.6 mg (0.039 mmol) and 13.2 mg (0.078 mmol of MCA in 2 ml of 0.5 M potassium phosphate buffer solution
- Reactions were carried out at pH 6.0 and 7.4 at 37°C

c) Formation of 3-(beta-D-Ribofuranosyl)7-formyl-8-[9'-(beta-D-ribofuranosyl)-N8-adenonsinyl]imidazo[2,1-i]purine (epsiloncA,A) by decarboxylation of 3-(beta-D-Ribofuranosyl)7-oxalo-8-[9'-(beta-D-ribofuranosyl)-N8-adenonsinyl]imidazo[2,1-i]purine (epsilonoA,A)
- 6 µg (epsiloncA,A) in 200 µl 0.5 M potassium phosphate buffer (pH 7.4) was held at 90 °C for 4 h

Test substance: other TS: Mucochloric acid, purity: >= 98% (source Fluka); see 1.1.1

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments

Flag: Critical study for SIDS endpoint

02-DEC-2003 (88)

Type: other: Reaction with Adenosine and Cytidine

Remark: The formation of the ethanocarbaldehyde derivatives and the previously identified etheno derivatives form mucochloric acid is explained by an initial conversion of mucochloric acid, through hydrolysis and decarboxylation to chloromalonaldehyde. Chloromalonaldehyde reacts with the nucleosides and forms an intermediate adduct which either undergoes ring closure by intramolecular displacement of the chlorine atom or breaks down to form chloroacetaldehyde which subsequently produces the etheno derivatives

Result: - Additional to the previous identified major product peak to be formed when MCA was reacted with adenosine or cytidine at

90°C pH 7 (see Kronberg L et al (1992) a second small peak was identified which eluted 4-5 min later
- Yield of this reaction product increased when reaction pH was 4.0
- peak was also identified in reaction of MCA with adenosine at 37 °C pH 7.4 but not in reaction of MCA with cytidine

PRODUCT IDENTIFICATION:
- Reaction with adenosine: ethenoadenosinecarbaldehyde [3-(beta-D-Ribofuranosyl)-7-formylimidazo[2,1-i]purine9
- Reaction with cytidine: ethenocytidinecarbaldehyde [6-(beta-D-Ribofuranosyl)-7-formylimidazo[2,1-c]pyrimidin-5-(6H)-one]

Test condition: ANALYSIS:
- Product isolation and sampling with HPLC
- HPLC1: Instrumed containing 2 Shimadzu LC-9A pumps and a Shimadzu SPD-6A UV spectrophotometric detector; detector at 290 nm
- HPLC2: HP 1090 equipped with diode-array detector

- Separation on Sperisorb ODS2 5 µm C18 reversed phase column (4 x 250 mm); isocratic elution for 5 min with 10% methanol in 0.01 M potassium dihydrogen phophate (pH 4.6) followed by a gradient from 10% to 30% methanol in 20 min at 1 ml/min
- Preparative isolation of products: on Nucleosil 7C18 semipreparative column 7 µm (10 x 250 mm); isocratic elution with 8% (adenosine reaction mixture) and 7.5 (cytidine reaction mixture) acetonitril in pure water
- 1HNMR and 13CNMR: JEOL GX-400 FT NMR spectrometer at 400 and 100 MHz respectively; samples dissolved in Me2SO4-d3; internal standard tetramethylsilane
- Assignment of carbon signals using proton-coupled and selectively proton-decoupled 13C NMR spectra
- Direct inlet electron impact (EI) mass spectra: VG 7070E mass spectrometer at 70 eV
- UV spectra: Shimadzu UV -160 spectrophotometer with diode array detector

REACTIONS WITH NUCLEOSIDES:
a) Reaction temperature: 90 °C for 24 h
- 8.2 mmol (1.38 g) MCA and 4.1 mmol (1.1 g) adenosine resp. 4.1 mmol (1.0 g) cytidine were added to 250 ml of a 0.5 M potassium phosphate buffer solution, adjusted to pH 4.0
- at the end of the reaction the reaction volume was reduced by rotary evaporation to approx. 80 ml
- reactions followed by HPLC separation and isolation
- collected fractions containing the products were rotary evaporated to dryness and residues subjected to spectrometric studies

b) Formation of etheno and ethnocarbaldhyde derivatives at various reaction conditions
- 0.08 mmol (13.1 mg) MCA was reacted with 0.04 mmol (10.7 mg) adenosine or 0.04 mmol (9.7 mg) cytidine in 2 ml of 0.5 M

potassium phosphate buffer solutions
- reactions carried out at 90 °C at pH 4.0, 6.0 and 7.4 and at 37 °C at pH 7.4

Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments

Flag: Critical study for SIDS endpoint

07-JUL-2003 (89)

Type: other: Reaction with Adenosine, Cytidine and Guanosine

Result:
- Reaction of MCA and Mucobromic acids with adenosine and cytidine in DMF resulted major product peaks which were identified as chloro- or bromopropenal derivatives respectively.
- Prenal derivatives of MCA with adenosine and cytidine are formed in DMF (yields of 18.5 and 7.7% resp.) as well as to a much smaller amount in aqueous solutions (about 5 x 10E-3% each).
- The reaction mechanism was investigated by analysis of 13C-labelling, which showed that only the aldehyde carbon of the chloroprenal unit was labeled when reaction was performed in DMF while only the carbon in the formyl group was labeled

in the aqueous reaction.
Reaction of MCA with guanosine: Formation of only trace levels of products that were not further investigated

Test condition: ANALYSIS:
- Product isolation and sampling with HPLC
- HPLC1: Instrument containing 2 Shimadzu LC-9A pumps and a Shimadzu SPD-6A UV spectrophotometric detector; detector at 290 nm
- HPLC2: HP 1090 equipped with diode-array detector
- Separation on Spherisorb ODS2 C18 5µm (4 x 125 mm) analytical column; isocratic elution for 5 min with 5% acetonitrile in 0.01 M potassium dihydrogen phosphate (pH4.6) followed by a gradient from 5 to 30% in 25 at a flow rate of 1 ml/min
- Preparative isolation of products by column chromatography on a 2.5 x 10 cm column of preparative C18 bond silica grade (40 µm, Bondesil); equilibration with water followed by batchwise elution with 0%, 5%, 10% and 15% acetonitrile in water batch volume: 100 ml
- 1HNMR and 13CNMR: JEOL JNM-A500 Fourier transform NMR spectrometer at 500 and 125 MHz respectively; samples dissolved in Me2S04-d6; internal standard tetramethylsilane
- Determination of shifts and coupling constants of the multiplets of the proton signals in the ribose units of adenosine adducts based on first order approach; for cytidine adducts due to small shift differences and interproton couplings calculation of spectral parameters unsing PERCH program
- Direct chemical ionization (DCI) mass spectra: VG 7070E mass spectrometer; ionization gas methane
- UV spectra: Shimadzu UV -160 spectrophotometer

PREPARATION AND PURIFICATION OF 13C-MCA:
- according to the method of Franzén and Kronberg (1995) Tetrahedron Lett 36:3905-3908

REACTIONS WITH NUCLEOSIDES:

a) Reaction with adenosine
- 1.19 mmol (200 mg) MCA was reacted with 0.59 mmol (159 mg) adenonsine in 8 ml of DMF for 3 days at 37 °C
- alternatively 1.19 mmol (200 mg) 13C-MCA (15 mol%) was reacted with 0.59 mmol (159 mg) adenonsine in 8 ml of DMF for 3 days at 37 °C

b) Reaction with cytidine
- 1.19 mmol (200 mg) MCA was reacted with 0.59 mmol (143 mg) cytidine in 20 ml of DMF for 5 days at 37 °C

c) Reaction with guanosine
- 1.19 mmol (200 mg) MCA was reacted with 0.59 mmol guanosine in 20 ml of DMF for 5 days at 37 °C

- after reactions solvent removal by rotary evaporation at 50 °C; residues dissolved in few ml water
- after filtering passage through the preparative C18 column

d) Small scale aqueous reaction with adenosine and cytidine
- 0.045 mmol (12 mg) adenosine resp. 0.045 mmol (11 mg) cytidine were reacted with 0.09 mmol (15 mg) MCA each in 2 ml of 0.5M phosphate buffer at pH 7.4 and 6.0 at 37 °C for 5 days

- determination of product formation by HPLC analysis of aliquots of the reaction mixtures

e) Aqueous reaction of 13C-3 labeled MCA with adenosine
- 0.32 mmol (85 mg) adenosine was reacted with 0.61 mmol MCA mixed with 13C-MCA (102 mg in total; 13 mol% 13-C-MCA) in 0.5M phosphate buffer at pH 6.0 at 90 °C for 12 h
- After filtration isolation of the products by use of the preparative C18 column as described above
- Upon evaporation of the fractions containing the products the compounds were crystallized; recrystallization was performed from warm water

QUANTIFICATION OF PRODUCT YIELDS:
- Quantitative 1H NMR analysis using 1,1,1-trichloroethane as an internal standard was performed on aliquots of the adducts
- Preparation of standard solutions for HPLC analysis by taking of exact volumes of the NMR samples and diluting them with appropriate volumes of water

- Quantitative determination of adducts in the reaction mixtures by comparing the peak area of the adducts at 330 nm in the standard solution with the area of the adduct peak in the reaction mixtures
- Calculation of the molar yields from the original amounts of adenosine or cytidine in the reaciton mixture

Test substance: no data

Conclusion: Based on the result of this study the formation pathway suggested for etheno and ethenocarbaldehyde derivatives as described in Kronberg et al. (1992) Chem. Res. Toxicol. 5: 852-855 was revised by the authors
The now suggested pathway for the formation of the chloroprenal derivatives, ethanocarbaldehyde derivatives and etheno derivatives from mucochloric acid in aquesous solutions is explained by an initial formation of mucoxychloric acid, which may be further broken down to chloractaldehyde, which could proceed via the chloromalonaldehyde that reacts with the nucleosides and forms subsequently the derivatives.

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments

Flag: Critical study for SIDS endpoint

10-JUL-2003 (90)

Type: other: Reaction with Adenosine, Cytidine, Guanosine and Uridine

Result: Either at 90 °C or at 37 °C one major product peak

REACTION WITH CYTIDINE:
- at 90 °C MCA consumed after 24 h reaction time
- product peak 3-5 min later than unmodified nucleosides
- at 37 °C, pH 7.0 product peak at the same retention time after 7 days reaction time
- product peak at 37 and 90°C identical and identified as 3,N4-ethenocytidine
- additionally poorly retained hydrophoilic compounds were formed in the reactions, partly due to MCA degradation in water

REACTION WITH ADENOSINE:
- at 90 °C MCA consumed after 45 h reaction time
- product peak 3-5 min later than unmodified nucleosides
- at 37 °C, pH 7.0 product peak at the same retention time after 7 days reaction time
- product peak at 37 and 90°C identical and identified as 1,N6-ethenoadenosine
- additionally poorly retained hydrophoilic compounds were formed in the reactions, partly due to MCA degradation in water

REACTION WITH GUANOSINE:
- at 90 °C MCA consumed after 45 h reaction time
- product peak 3-5 min later than unmodified nucleosides
- at 37 °C, pH 7.0 product peak at the same retention time after 7 days reaction time
- product peak at 37 and 90°C identical and identified as 1,N2-ethenoguanosine
- additionally poorly retained hydrophoilic compounds were formed in the reactions, partly due to MCA degradation in water

REACTION WITH URIDINE:
- no observable reaction between MCA and uridine

Test condition: ANALYSIS:
- Product isolation and sampling with HPLC
- HPLC1: Instrument containing 2 Shimadzu LC-9A pumps and a Shimadzu SPD-6A UV spectrophotometric detector; detector at 290 nm
- HPLC2: HP 1090 equipped with diode-array detector
- Separation on C18 reversed phase columns
- 1HNMR and 13CNMR: JEOL GX-400 FT NMR spectrometer at 400 and 100 MHz respectively; samples dissolved in DMSO-d3 (containing a few percent of CDCl3); internal standard tetramethylsilane
- Homo- and heteronuclear shift correlation and NOE experiments: JEOL standard programs
- Direct chemical ionization (DCI) mass spectra: VG 7070E mass spectrometer; source temperature 200°C; emission current 0.5 mA; electron energy 100 eV; resolution 1000; ionization gas methane
- EI mass spectra: HP 5971A mass selective detector connected to a HP 5890 (series II) gas chromatograph
- UV spectra: Shimadzu UV -160 spectrophotometer

REACTIONS WITH NUCLEOSIDES:

a) Reaction temperature: 90 °C
- 8.75 mmol (1478.4 mg) MCA was added to 100 ml of a 0.05 M potassium phosphate buffer solution, pH 7 containing either 1,9 mmol cytidine, adenosine, guanosine or uridine.
- reactions followed by HPLC analysis: Column 7 µm (4 x 250 mm) Semipreparative Nucleosil 7 C18 column; separation: isocratic elution with 7% acetonitril in water

b) Reaction temperature: 37 °C
- 5 µmol (0.84 mg) MCA reacted with 0.5 µmol cytidine, adenosine, guanosine or uridine in 10 ml 0.05 M potassium phosphate buffer solution, pH 7
- reactions followed by HPLC analysis: Column 5 µm (4 x 250 mm) Spherisorb ODS2 C18 column;

- Separation: isocratic elution for 5 min with 5% acetonitrile in 0.05 M potassium dihydrogen phosphate (pH 4.6); followed by gradient from 5 to 15% acetonitrile in 15 min at 1 ml/min

Fractions:
- fractions containing product peaks were rotary evaporated to dyness; recristallization of the products from water (3,N4-ethenocytidine and 1,N3-ethenoguanosine) respectively from water/ethanol (1,N6-ethenoadenosine)

Test substance: other TS: Mucochloric acid, purity: 99% (source Sigma-Aldrich); see 1.1.1

Reliability: (2) valid with restrictions
Study meets generally accepted scientific standards, well documented and acceptable for assessments

Flag: Critical study for SIDS endpoint

05-JAN-2004 (91)

Type: other: Toxicological Assessment

07-JUL-2003 (105)

6.1 Analytical Methods

-

6.2 Detection and Identification

-

7.1 Function

-

7.2 Effects on Organisms to be Controlled

-

7.3 Organisms to be Protected

-

7.4 User

-

7.5 Resistance

-

8.1 Methods Handling and Storing

Fire/Exp. Prot.: Ensure thorough ventilation of stores and work areas.
Storage Req.: Prevent from alkalies and alkali-forming substances.
Prevent from direct sunlight.
Add. Information: VCI - storage class: 8

Remark: Personal precautions: Prevent contact with skin, eyes and clothes

Environmental precautions: Do not let product enter drains.

Transport information

Land transport

ADR/RID	Class: 8	figure/letter: 65b
Warning panel	Hazard-no: 80	Substance no.: 1759

Inland waterway transport

ADR/ADNR	Class: 8	figure/letter: 65b

Sea transport

IMDG/GGVSee	Class: 8	UN-No.: 1759 PG: II
	EMS: 8-15	MFAG: 340

Proper technical name: Corrosive solid, n.o.s. (mucochloric acid)

Air transport

ICAO/IATA	Class: 8	UN/ID-No.: 1759 PG: II

Proper technical name: Corrosive solid, n.o.s. (mucochloric acid)

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

8.2 Fire Guidance

Prot. Equipment: Wear self-contained breathing apparatus and protective suit.
Ext. Medium: water, carbon dioxide (CO2), dry extinguishing media, foam
Add. Information: Collect separately contamined extinguishing water, do not allow to reach sewerage or effluent systems.
Dispose of fire debris and contaminated extinguishing water in accordance with local regulations.
Products arising: carbon monoxide, hydrogen chloride

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

8.3 Emergency Measures

Type: other: general advice

Remark: Immediately remove contaminated clothing.
First-aiders should pay attention to their own safety.
Flag: non confidential, Critical study for SIDS endpoint

19-NOV-2002 (1)

Type: injury to persons (skin)

Remark: Immediately wash thoroughly with plenty of water and soap. Consult a skin specialist.
Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

Type: injury to persons (eye)

Remark: Immediately wash affected eyes for at least 15 minutes under running water with eyelids held open, consult an eye specialist.
Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

Type: injury to persons (oral)

Remark: Immediately rinse mouth and then drink plenty of water, do not induce vomiting, summon physician.
Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

Type: injury to persons (inhalation)

Remark: keep patient calm, remove to fresh air, summon medical help.
Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

Type: accidental spillage

Remark: Environmental precautions: Do not let product enter drains. Methods for cleaning up/taking up: take up mechanically
Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

8.4 Possib. of Rendering Subst. Harmless

-

8.5 Waste Management

Memo: other: Product must be disposed of by special means, e.g. suitable incineration, in accordance with local regulations.

Flag: non confidential, Critical study for SIDS endpoint
19-NOV-2002 (1)

8.6 Side-effects Detection

-

8.7 Substance Registered as Dangerous for Ground Water

-

8.8 Reactivity Towards Container Material

-

(1) BASF AG, Safety data sheet MUCOCHLORSäURE TECHN., 15.10.2001

(2) Sigma-Aldrich (2003) Statement provided by technical service including excerpts of technical information system (unpublished data)

(3) MAK- und BAT-Werte-Liste 2002 (Mitteilung 38 vom 01.07.2002), WILEY-VCH Verlag GmbH, Weinheim, Germany

(4) Catalogue of Substances Hazardous to Water - Umweltbundesamt Berlin, status 11.11.2002

(5) National Chemical Inventories, 2002 Issue 1

(6) Hommel G (1992) Handbuch der gefaehrlichen Gueter, Merkblatt 1284, Springer-Verlag, Berlin Heidelberg, S. 1992

(7) BASF (1970) Safety data sheet Mucochlorsaeure, D 034, July 1970, BASF AG

(8) BASF (1976) Sicherheitstechnische Kenndaten, TLM/SIK 75/1379, 21.05.1976

(9) BASF (1999) Safety data sheet Mucochlorsaeure techn. rein, 06.01.1999, BASF AG

(10) BASF (2002) Calculation of vapour pressure with MPBPWIN v 1.40. BASF AG, Product Safety, Regulations, Toxicology and Ecology 19.07.2002 (unpublished data)

(11) BASF (1988) Partition coefficient, report to J.Nr.124837/01. BASF AG, Analytical Laboratory, 03.03.1988 (unpublished data)

(12) BASF (1989) Partition coefficient. BASF AG, Analytical laboratory, 09.01.1989 (unpublished data)

(13) BASF (1999) Sicherheitstechnik, interne Mitteilung (internal note), BASF AG, 05.11.1999

(14) Serjeant EP, Dempsey B (1979) Ionisation constants of organic acids in aqueous solution. IUPAC Chemical Data Series No. 23, Pergamon Press, Oxford, 1st ed., p. 81

(15) BASF (2003) Calculation of photodegradation in air. BASF AG, Department of Ecology, 05.08.2003 (unpublished data)

(16) BASF (1998) Department of Ecology, 20.12.1998 (unpublished data)

(17) Hemming J, Holbom B, Reunanen M and Tikkanen L (1986). Determination of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in chlorinated drinking and humic waters. Chemosphere 15, 549-556

(18) Kronberg L and Franzén R (1993). Determination of chlorinated furanones, hydroxyfuranones, and butenedioic acids in chlorine treated water and in pulp bleaching liquor. Environ. Sci. Technol. 27, 1811-1818

(19) Smeds A, Vartiainen T, Mäki-Paakkanen J and Kronberg L (1999). Concentration of Ames mutagenic chlorohydroxyfuranones and related compounds in drinking waters. Environ. Sci. Technol. 31, 1033-1039

(20) Suzuki N and Nakaniski J. (1990). The determination of strong mutagen 3-chlor-4-(dichloromethyl)-5-hydroxy-2(5)-furanone in drinking water in Japan. Chemosphere 21, 387-392

(21) Wright JM; Schwartz J, Varianinen T, Maki-Paakkanen J, Altshul L, Harrington JJ and Dockery DW (2002). 3-Chloro-4-(dichlormethyl)-5-hydroxy-2(5H)-furanone (MX) and mutagenic activity in Massachusetts drinking water. Environ. Health. Persp. 110: 157-164

(22) BASF (2002) Calculation of Mackay Level I v0.9 with BASF programme. Maisch R, BASF AG (1999) (programme developer) 22.07.2002 (unpublished data)

(23) BASF (2002) Calculation of Henry's law constant with HenryWIN v 3.10. BASF AG, Product Safety, Regulations, Toxicology and Ecology 18.07.2002 (unpublished data)

(24) BASF (2002) Calculation of Mackay Level I with Trent University programme v2.11. BASF AG, Product Safety, Regulations, Toxicology and Ecology 23.07.2002 (unpublished data)

(25) BASF (2002) Calculation of soil sorption coefficient with PCKOCWIN v 1.66. BASF AG, Product Safety, Regulations, Toxicology and Ecology 24.07.2002 (unpublished data)

(26) BASF (1981) Zahn-Wellens test (mucochloric acid; adapted inoculum). BASF AG, Dpt. of Ecology (unpublished raw data of 11.06.1981 + unpublished reprint of 05.07.2002)

(27) BASF (1981) Zahn-Wellens test (mucochloric acid; adapted inoculum). BASF AG, Dpt. of Ecology (unpublished raw data of 14.08.1981 + unpublished reprint of 05.07.2002)

(28) BASF (1981) Respirometric test (mucochloric acid purified technical grade). BASF AG, Dpt. of Ecology, 04.08.1981 (unpublished data; Reprint 18.07.2002)

(29) BASF (1981) Respirometric test (mucochloric acid purified technical grade; adapted inoculum). BASF AG, Dpt. of Ecology, 21.09.1981 (unpublished data; Reprint of 18.07.2002)

(30) BASF (1982) Zahn-Wellens test (mucochloric acid). BASF AG, Dpt. of Ecology, 03.02.1982 (unpublished data; Reprint 05.07.2002)

(31) BASF (1981) Zahn-Wellens test (mucochloric acid). BASF AG, Dpt. of Ecology, 14.04.1981 (unpublished data; Reprint 05.07.2002)

(32) BASF (1981) Zahn-Wellens test (mucochloric acid). BASF AG, Dpt. of Ecology, 10.12.1981 (unpublished data; Reprint

05.07.2002)

(33) BASF (1981) Zahn-Wellens test (mucochloric acid). BASF AG, Dpt. of Ecology, 07.05.1981 (unpublished data; Reprint 05.07.2002)

(34) BASF (1981) Biochemical oxygen demand in 5 days (BOD5) (mucochloric acid). BASF AG, Dpt. of Ecology, 04.05.1981 (unpublished data; Reprint 16.07.2002)

(35) BASF (2002) Calculation of BCF with Bcfwin v2.14. BASF AG, Product Safety, Regulations, Toxicology and Ecology 12.07.2004 (unpublished data)

(36) BASF (1988) Acute toxicity to Leuciscus idus (88/16). BASF AG, Department of Toxicology, 09.09.1988 (unpublished data)

(37) Isomaa, B. et al. (1995): The toxicity of the mutagen "MX" and its analogue, mucochloric acid, to rainbow trout hepatocytes and gill epithelial cells and to Daphnia magna, Toxicology 100, 69 - 77

(38) BASF (1988) Acute toxicity to Daphnia magna (0040/88). BASF AG, Department of Ecology, 11.02.1988 (unpublished data)

(39) BASF (1988) Toxicity to Scenedesmus subspicatus (0040/88). BASF AG, Department of Ecology, 10.06.1988 (unpublished data)

(40) BASF (1988) Toxicity to Pseudomonas putida (0040/88). BASF AG, Department of Ecology, 01.03.1988 (unpublished data)

(41) BASF (1981) Short-term respiration inhibition test with BASF activated sludge. BASF AG, Dpt. of Ecology, 07.04.1981 (unpublished data)

(42) BASF (1961) Acute toxicity and skin/eye irritation (X/211; X/329). BASF AG, Department of Toxicology, 13.02.1961 (unpublished data)

(43) Eastman Kodak Co. (1950) Toxicity report mucochloric acid, August 8, 1950 (submitted to OTS by Dupont Chem. Co. with cover letter dated 15.10.1992). Eastman Kodak Co., Laboratory of Industrial Medicine (unpublished data)

(44) LaLonde RT, Xie S, Bu L (1993). Test of chiral recognition in the Salmonella typhimurium (TA 100) mutagenicity of mucochloric acid-cysteine adducts. Environ Mol Mutagen 22: 181-187.

(45) LaLonde RT, Xie S (1992) A study of inactivation reactions of N-acetyldcysteine with mucochloric acid, a mutagenic product of the chlorination of humic substances in water Chem Res Toxicol 5: 618-624

(46) LaLonde RT, Xie S, Chamulitrat W, Mason RP (1994) Oxidation and radical intermediates associated with the glutathione conjugation of mucochloric acid. Chem Res Toxicol 7: 482-486

(47) LaLonde RT, Xie S (1993). Glutathion and N-Acetylcysteine inactivations of mutagenic 2(5H)-furanones from the

chlorination of humics in water

(48) BASF (1960) Acute toxicity and skin/eye irritation (X/211). BASF AG, Department of Toxicology, 09.09.1960 (unpublished data)

(49) BASF (1964) Acute toxicity in rats (XIV/182-184). BASF AG, Department of Toxicology, 22.09.1964 (unpublished data)

(50) Mashkina OH, Bathisina GS (1971) Toxicology and hygienic standards for mucochloric acid in the air of a working zone. Gig Tr Prof Zabol 6: 139-143 (in Russian with English abstract)

(51) Marhold J (1986) Prehled Prumyslove Toxikologie; Organicke Latky 558, Prague

(52) RTECS (2001) Registry of Toxic Effects of Chemical Substances. RTECS No. AS7700000, Update through 2001/01

(53) BASF (1960) Acute toxicity and skin/eye irritation (X/221). BASF AG, Department of Toxicology, 09.09.1960 (unpublished data)

(54) BASF (1980) Acute 4-hour inhalation toxicity in rats (77/164). BASF AG, Department of Toxicology, 24.12.1980 (unpublished data)

(55) BASF (1977) Acute dermal toxicity in rabbits (77/164 acute dermal toxicity). BASF AG, Department of Toxicology, 16.08.1977 (unpublished data)

(56) Romancik VE (1976). Hygienic-toxicological aspects in the manufacture of Burex and its intermediates. II. Observation of the effects of mucochloric acid on the organism. Cesk. Hyg. 21 (6-7), 319-325.

(57) BASF (1980) Corrosivity in rabbits (77/164). BASF AG, Department of Toxicology, 06.05.1980 (unpublished data)

(58) BASF (1964) Skin irritation in rabbits (XIV/97; XIV/182-184). BASF AG, Department of Toxicology, 13.08.1964 (unpublished data)

(59) BASF (1964) Skin irritation in rabbits (XIV/235-239; 247). BASF AG, Department of Toxicology, 01.10.1964 (unpublished data)

(60) BASF (1964) Skin sensitization in guinea pigs (XIV/97; XIV/182-183). BASF AG, Department of Toxicology, 15.09.1964 (unpublished data)

(61) Patty FA (1967) Industrial Hygiene and Toxicology. 2nd rev. ed., Vol. II, Interscience Publ., New York, p. 1975-1979

(62) Goldmann PJ (1972) Allergene und Hautkrankheiten in der werksärztlichen Praxis eines chemischen Industriebetriebes. Arbeitsmed Sozialmed Arbeitshyg 7: 98-100

(63) BASF (2001) Prenatal developmental toxicity study in Sprague-Dawley rats after oral administration, Report No.:

30R0345/999062. BASF AG, Product Safety, 12.11.2001 (unpublished data)

(64) Innes JRM, Ulland BM, Valerio MG, Petrucelli L, Fishbein L, Hart ER, Palotta AJ, Bates RR, Falk HL, Gart JJ, Klein M, Mitchell I, Peters J (1969) Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J Nat Canc Inst 42: 1101-1114

(65) NCI (1968) Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrial chemicals. National Cancer Institute, Bethesda, Maryland, USA (PB-223159)

(66) BASF (1981) Report on the study of Mucochlorsaeure techn. trocken (80/408) in the Ames test. BASF AG, Department of Toxicology, 14.07.1981 (unpublished data)

(67) BASF (1985) Report on the study of Mucochlorsaeure techn. rein (84/212) in the Ames test. BASF AG, Department of Toxicology, 13.05.1985 (unpublished data)

(68) Fekadu K, Parzefall W, Kronberg L, Franzen R, Schulte-Hermann R, Knasmueller S (1994) Induction of genotoxic effects by chlorohydroxyfurane, byproducts of water disinfection, in E. coli K.12 cells recovered from various organs in mice. Environ Molec Mutagen 24: 317-324

(69) BASF (1983) Mutagenicity evaluation of Mucochlorsaeure in the mouse lymphoma forward mutation assay (82/259). BASF AG, Department of Toxicology, 02.08.1983 (unpublished data)

(70) Jansson K, Hyttinen JMT, Niittykoski M, Mäki-Paakkanen J (1995) Mutagenicity in vitro of 3,4-dichloro-5-hydroxy-2(5H)-furanone (mucochloric acid), a chlorine disinfection by-product in drinking water. Environ Molec Mutagen 25: 284-287

(71) BASF (2001) In vitro micronucleus assay with mucochloric acid techn. pure in V79 cells, Report No.: 33M0345/994185. BASF AG, Product Safety (unpublished data)

(72) BASF (1985) Report on the study of Mucochlorsaeure (84/342, 343) in the Ames test. BASF AG, Department of Toxicology, 07.06.1985 (unpublished data)

(73) Ishiguro Y, LaLonde R, Dence C, Santodonato J (1987) Mutagenicity of chlorine substituted furanones and their inactivation by reaction with nucleophiles. Environ.Toxicol.Chem. 6: 935-946

(74) Ishiguro Y, Santodonato J, Neal MW (1988) Mutagenic potency of chlorofuranones and related compounds in Salmonella. Environ Mol Mutagen 11: 225-234

(75) Rapson WH, Nazar MA, Butsky VV (1980) Mutagenicity produced by aqueous chlorination of organic compounds. Bull Environ Contam Toxicol 24: 590-596

(76) Fouillet X, Gutty D, BarretJ (1983) Report on a study of the mutagenic potential of mucochloric acid (Ames test).

Battelle, Geneva Res. Centres, Carouge-Geneva, Switzerland; sponsor: Rhone Poulenc

(77) Meier JR, Ringhand HP, Coleman WE, Schenck KM, Munch JW, Streicher RP, Kaylor WH, Kopfler FC (1986) Mutagenic by-products from chlorination of humic acid. Environ Health Persp 69: 101-107

(78) LaLonde RT, Perakyla H, Cook GP, Dence CW (1990) Contribution of the 5-hydroxyl group to the mutagenicity of mucochloric acid. Environ Toxicol Chemistry 9: 687-691

(79) Hyttinen JMT, Niittykoski M, Jansson K (1995) Lack of uniformity in the mutational spectra of chlorohydroxyfuranones in Salmonella typhimurium strain TA100. Mutagenesis 10: 321-323

(80) Franzen R (1995) Synthesis and analyses of mutagenic chlorohydroxyfuranones and related compounds: by-products of chlorine disinfection of drinking water and of chlorine bleaching of pulp. Acta Academiae Aboensis Ser. B 55: 1-52

(81) LaLonde RT, Ramdayal F (1997) Mucochloric acid action on phi-x174 DNA: a comparison to other chlorine-substituted 2(5H)-furanones. Chem Res Toxicol 10: 205-210

(82) Maeki-Paakkanen J, Laaksonen M, Munter T, Kronberg L, Komulainen H (2001) Comparable DNA and Chromosome Damage in Chinese Hamster Ovary Cells by Chlorohydroxyfuranones. Environ Mol Mutagen 38: 297-305

(83) Kronberg L and Franzén R (1993). Determination of Chlorinated Furanones, Hydroxyfuranones and Butenedioic Acids in the Chlorine-Treated Water and in Pulb Bleaching Liquor. Environ. Sci. Technol. 27: 1811-1818.

(84) Le Curieux F, Nesslany F, Munter T, Kronberg L, Marzin D (1999) Genotoxic activity of chlorohydroxyfuranones in the microscale micronucleus test on mouse lymphoma cells and the unscheduled DNA synthesis assay in rat hepatocytes. Mutagen 14: 457-462

(85) Bodes E, Nakamura J, Swenberg JA (2003) SOT Annual Meeting Abstract No. 994.

(86) Le Curieux F, Munter T, Kronberg L (1997) Identification of adenine adducts formed in reaction of calf thymus DNA with mutagenic chlorohydroxyfuranones found in drinking water. Chem Res Toxicol 10: 1180-1185

(87) LaLonde RT, Xie S, Bu L (1993). Test of chiral recognition in the Salmonella typhimurium (TA 100) mutagenicity of mucochloric acid-cysteine adducts. Environ Mol Mutagen 22: 181-187

(88) Asplund D, Konberg L, Sjöholm R, Munter T (1995). Reaction of mucochloric acid with adenosine: formation of 8-(N6-adenosinyl)ethenoadenosine derivatives. Chem Res Toxicol 8: 841-846

(89) Kronberg L, Karlsson S, Sjöholm R (1993). Formation of

ethenocarbaldehyde derivatives of adenosine and cytidine in reactions with mucochloric acid. Chem. Res. Toxicol. 6: 495-499.

(90) Kronberg L, Asplund D, Mäki J, Sjöholm R (1996) Reaction of mucochloric and mucobromic acids with adenosine and cytidine: formation of chloro- and bromopropenal derivatives. Chem Res Toxicol 9: 1257-1263

(91) Kronberg L, Sjöholm R, Karlsson S (1992) Formation of 3,N4-ethenocytidine, 1,N6-ethenoadenosine, and 1,N2-ethenoguanosine in reactions of mucochloric acid with nucleosides Chem Res Toxicol 5: 952-855

(92) Daniel FB, Olson GR, Stober JA (1991): Induction of gastrointestinal tract nuclear anomalies in B6C3F1 mice by 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and 3,4-(dichloro)-5-hydroxy-2(5H)-furanone, mutagenic byproducts of chlorine disinfection. Environ Mol Mutagen 17: 32-39

(93) Fleig I, Zober A (1989) The use of cytogenetic monitoring in occupational medicine: a summary of choromosome analyses. Poster presented at: Biomonitoring and carcinogen risk assessment, Queen's College, Cambridge, England, 27-28 July 1989

(94) Bodes E, Nakamura J, Swenberg JA (2003) SOT Annual Meeting. Abstract No. 994.

(95) Steffensen IL, Paulsen JE, Alexander J (1997) Induction of aberrant crypts in mouse, but not in rat colon by mucochloric acid, a genotoxic hydroxyfuranone found in chlorinated drinking water. Proc American Assoc Cancer Res. Carcinogenesis 38: 469

(96) Steffensen IL, Paulsen JE, Engeset D, Kronberg L, Alexander J (1999) The drinking water chlorination by-products 3,4-dichloro-5-hydroxy-2[5H]-furanone (mucochloric acid) and 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone do not induce preneoplastic or neoplastic intestinal lesions in F344 rats, Balb/cA mice or C57BL/6J-Min mice. Pharmacol Toxicol 85: 56-64

(97) Kolesar D, Romancik V (1974) Das klinische und das Laborbild akuter Schaedigungen durch Zwischenprodukte des Herbicides Burex. Bratisl Lek listy 64: 132-140 (in Czech with German abstract)

(98) BASF (1961) Bacteriostatic and virostatic effect of mucochloric acid (X/211; 329). BASF AG, Department of Toxicology, 10.02.1961 (unpublished data)

(99) Woo YT, Lai D, McLain JL, Manibusan MK, Dellarco V (2002) Use of mechanism-based structure-activity-relationship analysis in carcinogenic potential ranking for drinking water disinfection by-products. Environ Health Persp Vol 110: Suppl 1

(100) Tuppurainen K and Lötjönen S (1993) On the mutagenicity of MX compounds. Mutat Res 287: 235-241

(101) Tuppurainen K, Lötjönen S, Laatikainen R, Vartiainen T (1992) Structural and electronic properties of MX compounds related to TA 100 mutagenicity. A semi-empirical molecular orbital QSAR study. Mutat Res 266: 181-188

(102) Tuppurainen K (1997) A plausible mechanism for the mutagenic activity (Salmonella typhimurium TA 100) of MX compounds: a formation of CG-CG+·-CG radical cation by one-elctron reduction. SAR QSAR Environ Res 7: 281-286

(103) LaLonde RT, Leo H, Perakyla H, Dence CW, Farrell RP (1992) Associations of the bacterial mutagenicity of halogenated 2(5H)-furanones with their MNDO-PM3 computed properties and mode of reactivity with sodium borohydride. Chem Res Toxicol 5: 392-400

(104) LaLonde RT, Perakyla H, Cook GP, Dence CW (1990) Contribution of the 5-hydroxyl group to the mutagenicity of mucochloric acid. Environ Toxicol Chem 9: 687-691

(105) BG Chemie (1997) Mucochlorsaeure, Toxikologische Bewertung Nr. 258. Berufsgenossenschaft der chemischen Industrie, Heidelberg