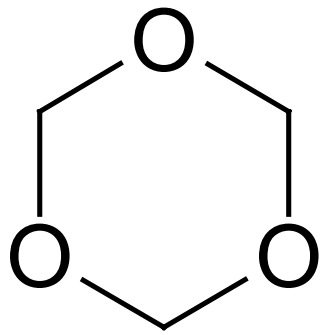

1,3,5-TRIOXANE

CAS Number 110-88-3

USEPA HPV Challenge Program Submission

December X, 2000

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Executive Overview

1,3,5-Trioxane (Trioxane) is a stable cyclic triether used primarily as a monomer for production of high-molecular weight polyacetals and secondarily as a chemical intermediate. Its production volume exceeds one million pounds per annum. It is estimated that over 99% of Trioxane production is used on the production sites as a monomer for making polyacetals. Polyacetals are strong and rigid resins that replace metals in many engineering applications. They are of little toxicological concern due to low bioavailability. FDA has approved polyacetals for food contact use and NSF has approved them for material in contact with potable water. Less than 0.2% of manufactured Trioxane is used in applications other than as a monomer. Exposure in these applications is limited by process controls, protective equipment and consumption in the application. Environmental releases are limited to vapor released to air at manufacturing sites.

The physical-chemical properties of Trioxane are well defined. It is a volatile solid at room temperature with a vapor pressure of 10 mm Hg. Trioxane is biodegradable in a waste water treatment facility but not considered readily biodegradable. Trioxane was found to have high hydrolytic stability in water at pH 4 to 9 with an estimated hydrolytic half-life at 25˚ C greater than one year. Vapors have an estimated photolytic half-life in air of approximately 25 hours, and predicted values for fugacity have been calculated with the MacKay model. Fish, daphnia and green algae are acutely affected by Trioxane only at concentrations greater than 2000 mg/l. Acute toxicity to mammals has been determined by oral, inhalation and dermal routes of exposure. Trioxane demonstrates a low order of toxicity with an oral LD_{50} of about 8000 mg/kg in rats. Repeated-dose studies have been conducted by the oral or the inhalation route with some published studies using exposures up to twelve months duration. A NOEL of 200 mg/kg/day has been reported for a 28-day oral gavage exposure study in rats. Genotoxicity has been evaluated using multiple *in vitro* and *in vivo* experimental procedures covering both mutation and chromosomal aberration. The weight of evidence indicates a lack of significant genotoxic properties. Lack of significant reproductive toxicity can be predicted based on an ovarian function (estrous cycle duration) study, and two dominant lethal studies that determined testicular pathology in conjunction with fecundity and offspring parameters. Developmental toxicity results are available and, although fetotoxicity can be produced at high levels, this occurs only at maternally toxic doses. Trioxane, therefore, is not considered a specific developmental toxin. ADME studies indicate that Trioxane is readily absorbed from the gastrointestinal tract and is rapidly metabolized primarily to carbon dioxide. Although most of the ingested Trioxane is rapidly metabolized and excreted, some appears to become incorporated into tissue probably by way of the C-1 metabolic pool.

With regard to the HPV program, no additional testing is proposed for Trioxane as all required parameters are sufficiently well characterized by available data.

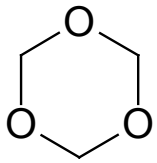
Testing Plan and Rationale

Testing Plan in Tabular Format

CAS Number 110-88-3 1,3-Trioxane	Information Available?	OECD Study ?	GLP Study?	Other Study ?	Estimation Method?	Acceptable?	Testing Recommended?	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	N	N	Y	N	
Boiling Point	Y	N	N	N	N	Y	N	
Vapor Pressure	Y	N	N	Y	N	Y	N	
Partition Coefficient	Y	Y	N	Y	N	Y	N	
Water Solubility	Y	N	N	N	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	N	Y	Y	N	
Water Stability	Y	Y	Y	N	N	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	Y	Y	N	Y	N	Y	N	
Ecotoxicity								
96-Hour Fish	Y	Y	N	Y	N	Y	N	
48-Hour Invertebrate	Y	Y	N	Y	N	Y	N	
72-Hour Algae	Y	Y	N	Y	N	Y	N	
Toxicity								
Acute	Y	N	N	Y	N	Y	N	
Repeated Dose	Y	Y	N	Y	N	Y	N	
Genetic Toxicology *in vitro*	Y	N	N	Y	N	Y	N	
Genetic Toxicology *in vivo*	Y	N	N	Y	N	Y	N	
Reproductive	Y	N	N	Y	N	Y	N	
Developmental	Y	Y	Y	Y	N	Y	N	

Introduction

1,3,5-Trioxane, CAS Number 110-88-3, (Trioxane) is a cyclic triether. It is a white stable solid with moderate water solubility used primarily as a monomer. Its structure is shown below:



The manufacturers estimate that over 99% of the production is used on the production sites as a monomer for making polyacetals. Polyacetals are very high molecular weight compounds of little toxicological concern. They are strong and rigid resins that can replace metals in many engineering applications. Polyacetals are used in a wide variety of applications, especially where low-friction properties are important. The polymer contains a very low level of free unpolymerized Trioxane and consumer exposure represents a negligible risk. FDA has approved polyacetal for food contact use and NSF has approved it for materials in contact with potable water. Less than 0.01% of manufactured Trioxane is used as a co-solvent in a commercial (non consumer) application and less than 0.2% is used in any application other than as a monomer. Exposure in these applications is limited by process controls, protective equipment and consumption in the application.

A very small amount of the production had been used to produce fuel bars. As Trioxane is a flammable solid, material in small bar form is useful as a spot heat source. These bars were primarily used by the military for heating food when standard heating devices were unavailable. Currently, these bars are available through military surplus outlets and some companies selling camping and emergency supplies. A search of suppliers on the Internet revealed that all retailers of these fuel bars are selling military surplus materials. The manufactures believe that no new production is occurring or planned for this use. Fuel bar use was intended for the military and consumer use represents a potential uncontrolled exposure to a limited number of individuals and, as no new production of fuel bars is taking place, this potential exposure is self-limiting.

Although Trioxane is a volatile solid with a vapor pressure of 10 mm Hg at 20º C, little vapor exposure occurs. In the industrial setting, exposures are well controlled and air concentration is maintained at a low level in the workplace. As there are few sites of manufacture, the number of potentially exposed individuals in industry is small.

Numerous studies have been conducted on fate and toxicity of Trioxane. These studies are briefly reviewed in this rationale document describing how they meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (USEPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries.

Physical-chemical Data

Physical-chemical data for Trioxane are available from the literature and company sources.

Melting Point	64° C (1)
Boiling Point	114.5 ° C @ 759mm (1)
Vapor Pressure	10 mm @ 20° C (2)
Partition Coefficient	Log $K_{o/w}$ = -0.42 (3) Log $K_{o/w}$ = -0.47 (4)
Water Solubility	17.2 g/100 ml @ 18° C (1) 21.2 g/100 ml @ 25° C (1)

These properties indicate that Trioxane is a volatile solid with moderate water solubility. The value of the partition coefficient suggests that Trioxane will partition into water and has little potential for bioaccumulation.

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints.

Environmental Fate and Pathways

Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. An experimentally derived rate constant is listed in the APOWIN program. Using the default atmospheric hydroxyl radical concentration in APOWIN and the measured rate constant for reaction of Trioxane with hydroxyl radical, the estimated half-life of Trioxane vapor in air is approximately 25 hours (5).

Water stability has been determined for Trioxane using the Organization for Economic Cooperation and Development Guideline (OECD) 111 protocol. In this study, Trioxane was found to be hydrolytically stable in the pH 4 to 9 range at elevated temperatures for six days. The estimated half-life for Trioxane in water at 25˚ C in this pH range is greater than one year (6).

Theoretical Distribution (Fugacity) of Trioxane in the environment was estimated using the MacKay model with standard defaults in EPIWIN v 3.05. (7) The results for distribution using a model calculated $K_{o/c}$ (adsorption coefficient based on organic carbon content) of 0.152 are:

- Air 4.8 %
- Water 53.4 %
- Soil 41.6 %
- Sediment 0.09 %

Trioxane was found not to be readily biodegradable showing little degradation in 28 days using a MITI Test (8). (Ready biodegradation tests use low levels of bacteria and simulate a lake or river; inherent biodegradation tests use high levels of bacteria and simulate a waste-treatment plant.) Volatilization was found to be a significant mechanism of loss under aeration conditions. A standard Zahn-Wellens (OECD 302B) inherent biodegradation test suggested biodegradation was occurring but the relative contribution of volatilization was not determined (9). A modified Zahn-Wellens test was conducted using a closed system with sodium nitrate as the oxygen source. In this study, greater than 90% of the DOC was removed in 18 days while only minimal loss of DOC was observed in controls without bacterial innoculum (10). This indicates that Trioxane is effectively removed from wastewater at a treatment plant.

Available data indicate that Trioxane will enter the environment by sublimation to a vapor and by means of wastewater. In wastewater treatment, volatilization and biodegradation are competing processes. Release to surface water is expected to result in volatilization within a period of days and no bioaccumulation. Photochemical oxidation will result in the ultimate degradation of Trioxane to water and carbon dioxide

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints.

Ecotoxicity

Trioxane was found to have minimal acute toxicity to typical aquatic species. The LC_{50} (96 - hour) for freshwater fish was found to be 4030 mg/l. (LC means lethal concentration; thus, an LC_{50} is the concentration resulting in death of half the fish in a particular time period, in this case, 96 hours.) The LC_0 and the NOEC (no observed effect concentration) were found to be 2150 mg/l. The lowest concentration causing 100% mortality was 10000 mg/ml (11). This study closely followed the OECD 203 guideline, with the exception of the recommended species of fish. Supporting this study, a screening level study sponsored by Celanese in saltwater fish was conducted in which Sheepshead minnows (*Cyprinodon variegates,* 5 per group) were exposed to Trioxane at concentrations of 10000, 20000 and 30000 mg/l. In this study, the 96-hour LC_{50} was reported to be 16350 mg/l (12). Other support comes from modeling. The EPA ECOSAR model (a model developed by EPA to predict the aquatic toxicity of certain class of compounds) found in EPIWIN estimates the 96-hour LC_{50} for fish as 17000 mg/l (13).

Aquatic invertebrate toxicity was examined in two studies of *Daphnia magna*. In the first, which followed the OECD 202 guideline, the EC_{50} (48-hour) and EC_0 (48-hour) were greater than 1000 mg/l (14). The second study was a screening level study, in which Trioxane was tested at 5000, 10000, 15000, 20000 and 30000 mg/l. In this study, the 48-hour EC_{50} was reported to be 15200 mg/l (12). Other support for a low-level of toxicity comes from modeling. The EPA ECOSAR model estimates the 48-hour LC_{50} for daphnia as 15000 mg/l (13).

Toxicity to aquatic plants was evaluated in two studies. The first, conducted by BASF Corporation, measured the growth rate of *Scenedesmus subspicatus* in the presence of Trioxane at concentrations up to 500 mg/l. Little inhibition was observed and the EC_{50} was found to be greater than 500 mg/l (15). This study is supported by an earlier screening-level study, sponsored by Celanese, in which Trioxane was tested for growth inhibition of *Selenastrum capricornutum.* In this study, algae growth was measured out to 14 days of exposure at levels of 1000, 5000 or 10000 mg/l. Significant inhibition occurred only at 10000 mg/l; the reported NOEC was 5000 mg/l (12).

All the reported ecotoxicity studies used nominal concentrations of Trioxane and not analytically measured concentrations. There are several potential sources of error in using nominal concentrations. The most important of these are mistakes in sample preparation, loss of material by volatilization from solution, hydrolysis of test material and biodegradation during the exposure period. Consistency of findings across laboratories indicates reliability in preparation of exposure levels. Volatilization was also considered an insignificant source of error based on the following two analyses. Volatilization can be estimated from the inherent biodegradation study where an aerated sample without bacterial innoculum was used as control.

Under these conditions, the air stripping of DOC (dissolved organic carbon) was 17% at 2 days and 27% at 4 days (10). An alternative means of estimation comes from EPIWIN which estimates the half-life of Trioxane in a river with a 1 meter water depth, a 1 meter per second current (well mixed) and a wind velocity of 5 meters per second to be 90 hours. Based on the EPIWIN estimate, excessive volatilization of Trioxane is not anticipated during a 2-day (daphnia) or a 4-day (fish) test. The worst-case condition, where the sample is well mixed and there is rapid exchange of air over the surface, indicates a loss of 50% over the 4 days of a fish test. As there was no mortality at the 2150 mg/l level, Even with a 50% volatilization loss the LC_{50} still is in excess of 1000 mg/l.

Biodegradation can be eliminated as a means of significant test material loss since Trioxane is not readily biodegradable. Hydrolysis, likewise, has been shown not to be an important source of compound loss in this pH range. The concentration levels used in the fish tests and the supporting daphnia test (48-hour LC_{50} of 15,200 mg/l) were also far greater than the currently OECD acceptable maximum concentration limits of 100 mg/l for fish and 1000 mg/l for daphnia. Thus, concentration errors, volatilization, biodegradation and hydrolysis can be eliminated as factors that would significantly change the conclusion that Trioxane has a low level of acute toxicity to typical aquatic organisms. It can be concluded that the ecotoxicity tests are adequate for the purposes of defining these SIDS endpoints. The ECOSAR model also supports the observed low toxicity to aquatic organisms.

Recommendation: No additional testing is required. The ecotoxicity results indicate that Trioxane is of low concern to aquatic environmental species. Although the studies were not conducted with measured concentrations of Trioxane, the established volatility and high initial levels provide adequate assurance of low acute toxicity.

Acute Toxicity

Oral Exposure

The acute oral toxicity was determined in male rats using water as vehicle. The high dose, 10000 mg/kg, produced mortality in four of five animals. No mortality was observed at 5000 mg/kg or lower doses. Target organs were not identified and the LD_{50} (dose resulting in 50% mortality) was calculated to be 8190 mg/kg (16). This study is supported by a study in the literature that reports the acute oral LD_{50} to be 8500 mg/kg (17).

Inhalation Exposure

A well-documented guideline-like GLP study was conducted in 1986. The results appear reliable and show that the LC_{50} (inhalation concentration resulting in 50% mortality) for

Trioxane is greater than 10600 ppm for a 4-hour exposure. No target organs were identified. The high-dose was the maximum vapor concentration that could be achieved (18). This study is supported by a study in the literature that reports the acute inhalation LC_{50} to be greater than 6500 ppm (>26000 mg/m^3) for a four-hour exposure (17).

Dermal Exposure

Trioxane produced no mortalities when administered by the dermal route to four albino rabbits at a dose of 3980 mg/kg body weight. In this study, the only signs of toxicity were a reduction in weight gain during the first week post-exposure and slight to moderate skin irritation noted at 24 hours (19).

Recommendation: No additional acute studies are required. Trioxane has been evaluated for acute toxicity by all three of the usual routes of exposure and the results demonstrate that Trioxane has low potential for producing acute toxicity.

Repeat Dose Toxicity

Oral Exposure

In a 28-day gavage study, five rats of each sex per group were exposed to 0, 40, 200, or 1000 mg/kg/day Trioxane in water vehicle. High dose animals of each sex showed a significant decrease in leucocytes and there was a tendency for a decrease in leukocytes at the 200 mg/kg/day level in males which did not reach statistical significance. In addition, high-dose males were found to have an increase in serum gamma-glutamyl-transpeptidase and high-dose females were found to have increases in serum gamma-glutamyl-transpeptidase. High-dose females also displayed a decrease in serum protein and glucose. The 200 mg/kg/day level was considered a NOAEL. Adverse effects on the testes were reported in 1/5 high-dose males (20).

Inhalation Exposure

In a 1983 14-day study, rats were exposed to Trioxane at concentrations of 0, 103, 984 or 4940 ppm. Rats exposed to 4940 ppm Trioxane vapor displayed signs of toxicity including decreases in weight gain, righting reflex and grip strength. A dose-related increase in the incidence of splenic atrophy, decreases in spleen weights, and squamous metaplasia of the anterior nasal passage were observed at 984 and 4940 ppm. Animals exposed to 103 ppm showed a low incidence of rhinitis indicative of minor irritation and males of this group were found to have reduced spleen weights as compared to controls. The LOAEL (lowest observed adverse effect concentration) for males was considered to be 103 ppm, this level was considered a NOAEL for females (21).

There is good correlation between the oral and inhalation studies in terms of dose and target organ response. For example, both studies showed reduction in WBC's. In the oral study, this reduction was significant at 1000 mg/kg/day and for the inhalation dosing it was significant at 4950 ppm (equivalent to 4950 mg/kg/day total inhaled dose assuming a 270 ml minute volume for a 350 gram rat (22)). Splenic weight changes occurred at an oral dose of 1000 mg/kg/day and at inhalation doses of 984 ppm for females and 103 ppm for males (the 103 ppm dose was a NOAEL for all other parameters in the inhalation study). The NOAELs and LOAELs are compared in the table below.

Sex	Parameter	28-Day Oral (mg/kg/day)	14-Day Inhalation (mg/kg/day)*
Male	NOAEL	200	<105
	LOAEL	1000	105
Female	NOAEL	200	105
	LOAEL	1000	1005

* Assuming a 270 ml minute volume for a 350-gram rat, and 100% absorption, not correcting for days/week dosed or study duration.

From an examination of this table it appears that rats are slightly more sensitive to the adverse effects of Trioxane by inhalation than by gavage. If one considers that the inhalation absorption is probably less then 100% and that the inhalation study duration was about half that of the gavage study, inhalation appears to be the more sensitive route. It is speculated that after oral administration, first-pass metabolism by the liver detoxifies a portion of the Trioxane reducing the oral toxicity compared to inhalation. This possibility is supported by the metabolism data that indicate Trioxane is rapidly metabolized and primarily excreted as carbon dioxide from the lungs (see Metabolism and Special Studies).

Recommendation: No additional testing is required. The SIDS repeated-dose endpoint is filled by two studies of good reliability that cover the two important routes of exposure. Additional supporting data from longer-term oral and inhalation studies are also available (23, 24, see robust summaries of the repeated-dose studies for summary details of the longer term studies).

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points: generally one sensitive to point mutation and one sensitive to chromosomal aberrations. In the case of Trioxane, these requirements are fulfilled by multiple studies covering both types of end-points.

Genetic Toxicology in vitro

A *S. typhimurium* reverse mutation assay (BASF, 1988; robust summary attached) was conducted in four strains of bacteria using a triple plate, independent repeat design. It is supported by a single-plate test from another laboratory using five strains of bacteria (TA98, TA100, TA1535, TA 1537 and TA1538) exposed with and without S-9 activation at eight concentrations of Trioxane from 0.5 to 5000 micrograms per plate. No mutagenic response was recorded in this study (25). These studies show that the material is not genotoxic to *S. typhimurium* under these conditions.

Two additional studies in prokaryotes produced negative results In a triple plate test, with preincubation in the presence and absence of S-9 with strains TA97, TA98, TA100 and TA1535 at concentrations from 100 to 10000 micrograms per plate, Trioxane was reported to be without mutagenic activity (26). The mutagenic activity of Trioxane was investigated in five strains of *S. typhimurium*: TA1535, TA1537, TA1538, TA98 and TA100 with and without activation by liver microsomes (induced with Aroclor 1254). It displayed no mutagenic activity under any of these conditions (27).

A mouse lymphoma assay showed no genotoxic activity in the absence of metabolic activation; however, genotoxicity was indicated in the presence of metabolic activation. The dose-dependent increase in the number of mutants under activation conditions was associated with significant cytotoxicity at the higher dose levels (28).

A cell transformation study was conduced using C3H 10T-1/2 cells. A wide range of concentrations was tested using both 11-day and 38-day incubations following a 24-hour exposure to Trioxane. No increase in the number of transformed colonies or transformed foci was noted at any concentration of test material (29).

Genetic Toxicology in vivo

A published mouse micronucleus study showed no genotoxic activity in BALB/c mice. Mice were given Trioxane by intraperitoneal injection in two doses at 24-hr intervals. Intraperitoneal administration of Trioxane produced no chromosomal damage resulting in erythrocyte micronucleus formation, even at highly toxic doses (30).

Trioxane was tested for its ability to induce DNA repair (Unscheduled DNA synthesis; UDS) *in vivo* using rat hepatocytes. Trioxane, dissolved in water, was administered once orally to male Wistar rats at dose levels of 250 mg/kg, 500 mg/kg, 1000 mg/kg or 2000 mg/kg body weight. Treatment with test substance did not lead to an increase in the mean number of net nuclear grain counts from 500 mg/kg up to 2000 mg/kg body weight at any exposure time in rat hepatocytes. The lowest dose group (250 mg/kg body weight) was not evaluated since the two higher dose groups were negative. Under these test conditions, Trioxane was considered negative in the rat hepatocyte *in vivo* UDS assay. (31)

Two dominant lethal studies have been conducted in rats. An oral study was conducted in which male rats received Trioxane daily for eight weeks and effects on fertility were evaluated for in a weekly mating design. Oral doses of 0, 850 and 1700 mg/kg/day had no effect on male fertility indexes. Pregnancy outcomes were also comparable to controls. No dominant lethal effect was observed under these conditions (33).

The second dominant lethal study was an inhalation study in which male rats were exposed to Trioxane 5 hours daily, 5 days a week, for 12 months, at concentrations of 2500 mg/m^3. At the end of the 12-month exposure period, males were mated with females in a ratio of 1:2 through one week. Dams were sacrificed 13-14 days after the middle of mating intervals. No increase in the number of preimplantation losses, dead implants and live fetuses per female was noted in any treated group as compared to an appropriate control group. Trioxane did not affect the fertility of males. The study did not reveal induced dominant lethal mutations in germ cells from inhalation exposure to Trioxane (33).

Recommendation: No additional testing is required. The SIDS requirement for genetic testing has been fully met by a battery of several studies both *in vitro* and *in vivo* and sensitive to both mutations and chromosome aberrations. With the exception of the mouse lymphoma in the presence of metabolic activation, all studies demonstrated lack of genotoxic activity. The weight of evidence suggests low genotoxic potential for Trioxane.

Reproductive Toxicity

Trioxane has not been tested for reproductive toxicity using a standard reproductive testing protocol; however, several studies are available which supply the information necessary to assess potential reproductive effects. The SIDS Manual (32) gives guidance for the amount of information necessary to fill the toxicity to reproduction requirement in the absence of OECD guideline (OECD 415, 416, 421 or 422) studies. The SIDS manual indicates that the reproductive endpoint can be met alternatively by stating "*when a 90-day repeated-dose toxicity study is available and demonstrates no effect on reproductive organs, in particular the testes,*

then a developmental study (e.g. OECD Test Guidelines 414) can be considered adequate to complete information of reproduction/developmental effect." In the case of Trioxane, comprehensive and reliable long-term repeated dose studies are not available; however, testicular and ovarian functions were evaluated using other protocols and a reliable developmental toxicity study is available.

Testicular Function Tests

In a dominant-lethal study, male rats that received Trioxane daily for eight weeks were evaluated for effects on fertility in a weekly mating design. Oral doses of 0, 850 and 1700 mg/kg/day Trioxane (up to 20% of the LD_{50}) had no effect on male fertility indexes. Pregnancy outcomes were also comparable to controls. Although no functional deficit in fertility was recognized in these males, microscopic evidence of testicular degeneration and alterations in spermatogenesis were noted (33). Focal necrosis of the seminiferous epithelium was reported in 1/10 control, 3/10 850-mg/kg males and an unspecified number of high dose males. It was reported that the testicular lesions were bilateral in 3/10 high-dose males. Severity was reported as being dose dependent.

In a 12-month inhalation study (inhalation dominant-lethal study, robust summary attached), male rats were exposed to Trioxane 5 hours daily, 5 days a week, for 12 months, at concentrations of 2500 mg/m^3 (equivalent to 580 mg/kg/day assuming a 270 ml/min minute volume, a 350-gram rat and 100% absorption (22)). At the end of the 12-month exposure period, males were mated through one week with female rats in ratio 1:2. The autopsy of dams was carried out 13-14 days after the middle of mating intervals. No increase in the number of preimplantation losses, dead implants and live fetuses per female was noted in any treated group as compared to an appropriate control group. Trioxane did not affect the fertility of males, and no histopathological changes in the testes were reported. (It cannot be definitively determined that testes were examined macroscopically or microscopically; however, in the oral dominate-lethal included in the same publication, testicular effects by histopathologic examination were noted. Thus, it is anticipated that the testes were examined). The study did not reveal induced dominant lethal mutations in germ cells from inhalation exposure to Trioxane (33).

Ovarian Function Test

Starek and Barazski (34) studied the effect of Trioxane exposure on the estrous cycle of rats. In this study an aqueous solution of Trioxane was administered by gavage to female rats, 5 days per week for 7 weeks, at doses of 190, 580 or 1160 mg/kg/day. They reported that a significant increase in the mean duration of the estrous cycle, mainly due to lengthening of the diestrous, was only noted in the 6^{th} and 7^{th} week of compound administration at a dose of 1160 mg/kg. Three weeks after cessation of treatment, the cycle had returned to normal in the affected group. A statistically significant dose-related decrease in body weight gain was observed in females

given test material at all three treatment levels and Trioxane-induced behavioral changes were seen in the high-dose group. It was, therefore, concluded that exposure to Trioxane did not affect the sexual cycle unless other overt signs of toxicity were present. Histopathology or other measures of toxicity were not recorded and it is assumed they were not collected; however, estrous cycle activity is itself a strong indication that Trioxane does not interfere with ovarian function.

Results from this study correspond well with the 28-day gavage study (20) where a NOAEL of 200 mg/kg and a LOAEL of 1000 mg/kg were reported. In the ovarian function test, the 580 mg/kg dose group would have been the NOAEL (based on body weight) had the study been terminated at 28-days. It was only after six weeks of dosing that the body weights became significantly different for the 580 mg/kg group and only at the end of eight weeks dosing for the 190 mg/kg group.

The logical value of the estrous cycle assay as a sensitive indicator of female reproductive toxicity is high since it requires both ovarian and neuro-hormonal integrity. It is also supported by published literature. May and Finch (35) advocated estrous cycle as a method to test presumptive reproductive toxins noting that quantitative changes in cycle length are preferred. They stated that cycle length distributions indicate more subtle impairments in reproductive function than other available methods; however, they require more refined data analysis. Chapin et al. (36), reported on the relationship among reproductive endpoints in Swiss mice using the reproductive assessment by continuous breeding database, containing data for 72 chemicals at that time. In these studies, it was noted that longer estrous cycle time correlated well with reduced pup numbers. They concluded that estrous cycle length is a useful surrogate of overall reproductive function for females. The EPA has recently added estrous cycle length determinations to the multi-generation testing guidelines and noted, “The new endpoints for monitoring pubertal development, semen quality, and estrous cyclicity will better enable determination of the affected sex, target organ, and life stage following exposure throughout the life cycle.” (37).

Recommendation: No additional testing is required. The available data show effects on female reproductive function only at systemically toxic doses, and no functional deficits in male reproductive function after long-term dosing. Additional testing would not add significantly to the information necessary to define the reproductive toxicity for this material.

Developmental Toxicity

A developmental toxicity study for Trioxane was completed by Hoechst Marion Roussel (HMR) in 1998 using the OECD 414 guideline and also in accord with the appropriate EU and US EPA guidelines (38). In this gavage study, groups of pregnant rats were treated at 0, 100, 315 or 1000 mg/kg/day from days 7 to 20 of pregnancy. Results of this study indicate that Trioxane is not a specific developmental toxin. The developmental NOEL was found to be 100 mg/kg/day while a maternal NOEL was not defined since corrected dam body weight gain was affected in all treated groups. The higher dose levels, which were maternally toxic, produced numerous variations in the offspring indicative of fetotoxicity associated with the maternal toxicity. Two developmental toxicity studies of Trioxane have been published and these are summarized in the robust summary for the developmental toxicity study (39,40).

Recommendation: No additional testing is required. The HMR study meets the HPV requirements for detailed and well-documented developmental toxicity data and the results are generally supported by two published studies. When the data are examined as a whole, Trioxane, when dosed using the daily regime recommended in the OECD guidelines, only causes adverse developmental effects in the presence of maternal toxicity.

Metabolism and Special Studies

A study was conducted to investigate Trioxane distribution, excretion and metabolism. Male Wistar albino rats were administered a single dose of ^{14}C Trioxane at either 40 mg/kg or 400 mg/kg. Exhaled air was found to be the main route of ^{14}C elimination, which was mainly in the form of CO_2. Elimination of Trioxane by exhalation during the first 12 hr following the administration of 40 mg/kg was rapid with a half-life of 3.5 h. At 400 mg/kg, Trioxane was eliminated 77% as CO_2 and 8% as unchanged Trioxane in exhaled air. Also in this 12-hour period, about 3% of the ^{14}C was excreted in the urine in the form of unchanged Trioxane. This demonstrates that Trioxane is rapidly and extensively metabolized. A small portion of the administered Trioxane is removed more slowly and may become a part of the C-1 metabolism pool. Trioxane elimination from the plasma, at a dose of 40 mg/kg, showed biphasic elimination, with half-lives of 4.5 and 72 hours. The primary elimination route appears to be metabolism and excretion of carbon dioxide from the lungs. Distribution studies indicated that liver had the highest ^{14}C concentration of examined organs while fat tissue and brain had the lowest. The authors concluded that Trioxane is rapidly eliminated and is not expected to accumulate within tissue (41).

Another metabolism study of Trioxane was conducted by administering 2500 mg/kg ^{14}C-Trioxane to three Sprague-Dawley rats of each sex weighing between 221 and 246 grams. The radioactive Trioxane was administered by oral gavage as a water solution. Exhaled carbon

dioxide, urine and feces were collected for the intervals 0-12, 12-24, 24-48 and 48-72 hours. Radioactivity was determined in each matrix for each time interval. Overall, during the 72 hours after administration, ^{14}C elimination was about 72% in exhaled air as CO_2. About 15% was recovered in urine and less that 1% in feces. About 2% of the initial dose remained in tissues after 72 hours with the majority being found in the liver. Total ^{14}C recovery was about 90%. Mean data for radioactivity trapped in sodium hydroxide (presumed to be carbon dioxide) at each time interval for each group is given below.

	Percent of Total Radioactivity Exhaled as Carbon Dioxide	
Time Interval	Males (n=3)	Females (n=3)
0-12	23.3	16.1
12-24	26.7	25.0
24-48	9.1	11.6
48-72	0.7	0.8
Total	73.8	71.5

It was concluded that Trioxane is readily absorbed from the gastrointestinal tract and rapidly metabolized to CO_2; no sex difference was found in metabolism by these measures (42). Although a half-life was not calculated in this study, it is apparent from the data that most of the radioactive carbon dioxide was exhaled in the first 24 hours. More radioactive carbon dioxide was exhaled in the second twelve-hour period than the first. It can be speculated, based on the metabolic results at lower doses that this probably represents saturation of the metabolic pathways at this 2500 mg/kg dose. The lower carbon dioxide exhalation in the first twelve hours may be accounted for by a time lag between administration and absorption.

Studies were performed in an attempt to determine if Trioxane reacts hydrolytically with whole blood to form simpler species. Pooled control rat blood was incubated with Trioxane at 0, 10, 100 or 500 mg/l for one hour at 37º C. Recovery of unchanged Trioxane after the one-hour incubation was used as a measure of possible metabolism. The average recoveries of Trioxane were 73, 93 and 80%, respectively, for 10, 100 and 500 mg/l fortifications of a homogeneous sample of pooled control rat blood. The investigators concluded these results show that the majority of Trioxane incubated with whole blood for one hour could be recovered. This suggests that little, if any hydrolysis or metabolism occurs with blood alone (43).

A study in pregnant rats was conducted to determine tissue distribution and binding of ^{14}C activity at various time intervals following oral administration of radiolabeled Trioxane. A single oral dose of universally labeled ^{14}C-Trioxane at 40 mg/kg was administered to pregnant rats. Animals were killed on the 21st day of gestation 3, 24, or 48 hours after administration of Trioxane. In maternal rats, 3 hours after administration, the highest levels of total radioactivity

were found in liver and plasma, followed by a gradual decline with time. The level of ^{14}C-activity in the fetus was comparable to that of the maternal kidney throughout the study. The radioactivity in the fetal kidney and liver 48 hours after administration was higher than at 3 hours after administration. A slow decline in radioactivity was observed with time in the fetal brain, skin and carcass. After 48 hours, however, radioactivity in the fetal kidney and brain was more than twice as high as in the corresponding maternal organs. Three hours following ^{14}C-Trioxane administration, 35 and 41% of total radioactivity in maternal liver and kidney, respectively, was firmly bound to macromolecules, while the fetal liver and kidney showed 100 and 72% binding of radioactivity, respectively (44). The relative accumulation of radioactivity in fetal tissue is expected if Trioxane is metabolized and enters the C-1 pool, perhaps by way of tetrahydrofolate. The fetal tissues are undergoing rapid growth and incorporation of C-1 moieties as compared to maternal tissues. It could not be distinguished if Trioxane was binding to fetal tissues by means of a reactive metabolite or by orderly incorporation by way of the C-1 pool. It was established, however, that Trioxane is rapidly metabolized to carbon dioxide and C-1 pathways would be expected to saturate with radioactive carbon from Trioxane. Thus, C-1 pathways are considered likely to be responsible for much of the fetal incorporation of radioactive carbon.

These ADME studies conducted indicate that Trioxane is readily absorbed from the gastrointestinal tract and is metabolized primarily to carbon dioxide. Either it or a metabolite crosses the placenta and is incorporated into fetal tissues. Although most of ingested Trioxane is rapidly metabolized and excreted, some appears to become incorporated in tissue. With the available data, it cannot be distinguished definitively if a metabolite of Trioxane reacts with tissue macromolecules directly or if it enters the metabolic C-1 pool where it is incorporated into tissue, or both. Data from other metabolism studies implicate the C-1 pool mechanism as important in the incorporation of radioactive carbon from Trioxane in tissues.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, the available data fill all of the requirements for chemical parameters, fate and toxicity information. No additional studies are proposed for this program.

References

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Robust Summaries

Melting Point

Type	**Melting Point**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3

Method

- Guideline

• Test Type	Melting Point
• GLP	No
• Year	Unknown

Result

• Melting Point	64 deg C
Remarks Field for Results	Handbook data

Conclusions

Remarks field	Melting point is 64˚ C.

Data Quality

• Reliability	Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks.

References

1. The Merck Index. 9th ed. Rahway, New Jersey: Merck & Co., Inc., 1976. 1249

Boiling Point

Type	**Boiling Point**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3

Method

- Guideline

• Test Type	Boiling Point
• GLP	No
• Year	Unknown

Result

• Boiling Point	114.5 deg C @ 759 mm Hg
Remarks Field for Results	Handbook data

Conclusions

Remarks field	114.5 deg C @ 759 mm Hg

Data Quality

• Reliability	Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks.

References

1. The Merck Index. 9th ed. Rahway, New Jersey: Merck & Co., Inc., 1976. 1249

Vapor Pressure

Type	**Vapor Pressure**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3

Method

- Guideline
- Test Type — Vapor Pressure
- GLP — No
- Year — Unknown

Result

- Vapor Pressure — 10 mm Hg @ 20 deg C

Remarks Field for Results: Determined value

Conclusions

Remarks field: This material is a volatile solid at normal room temperatures. VP values for solids are not generally reported but, in this case, it is of value in risk assessment.

Data Quality

- Reliability — Klimisch Code 2. Physical measurement conducted by reliable laboratory, considered reliable although not conducted under GLP conditions.

References: Determination by Celanese Chemicals at the Corpus Christi Technical Center.

Other: This result is supported by the MPBPWIN v1.40 modeling program which uses three methods to estimate the VP based on the experimentally determined melting point and boiling point (both found in the EPIWIN data base) to estimate values of 11.8, 10.5 and 11.1 mm Hg @ 25˚ C. with 10.5 mm selected by the program as the preferred estimated value.

Reference for Supporting Studies: MPBPWIN v1.40 as found in EPIWIN v3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Partition Coefficient

Type **Partition Coefficient**

Test Substance 1,3,5-Trioxane
CAS Number: 110-88-3

Method

- Method: Shake flask
- Test Type: Partition Coefficient
- GLP: No
- Year: 1989

Remarks Field for Test Conditions

- ◊ Replicates: One replicate per test concentration
- ◊ Concentration: 55, 102, 551 mg test material
- ◊ Volumes: Water 25 to 35 ml; n-Octanol 25 ml
- ◊ Analysis: Triplicate GC analysis of each phase of each replicate
- ◊ Variation From Current OECD Guideline: This study used single replicates of three test material concentration in relatively fixed volumes of Octanol/water. The current (1995) OECD 107 guideline calls for duplicate runs at three solvent ratios and possibly test substance quantities. As the partition coefficient is near unity this deviation is considered to have minimal effect on the outcome.

Result

- Partition Coefficient: Log $K_{o/w}$ = -0.47
- Temperature: 25˚C

Remarks Field for Results: All three concentration of test material gave similar partition coefficients, they were Po/w of 0.36, 0.32 and 0.34 for test material quantities of 55, 102, 551 mg, respectively.

Conclusions

Remarks field: Log $K_{o/w}$ was determined to be -0.47 using the shake-flask method

Data Quality

- Reliability

Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although it could not be established that the study was conducted in accord with GLP standards. Study design and reporting meets current EPA/OECD guidelines with minor exceptions.

References

Reprint Of A Test Report "Determination of partition coefficient n-octanol/water of 1,3,5–Trioxane by shake flask method." Analytical report Number 107243/01 Test period: May 1989

Other

This result is supported by a literature value of -0.43[1] and by the the Log Kow v1.66 modeling program which estimates a value of –0.56 for Log Ko/w

Reference for Supporting Studies

1. Hansch. C., A. Leo and D. Hoekman. 1995. Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Professional Reference Book. Washington, DC: American Chemical Society as cited in Documentation for KOWIN estimation program version 4.00.5000, Syracuse Research Corporation, Syracuse, NY (April 2000)

2. Log Kow v1.66 as found in EPIWIN v3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Water Solubility

Type	**Water Solubility**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3

Method

- Guideline

• Test Type	Water Solubility
• GLP	No
• Year	Unknown

Result

• Solubility	◊ 17.2 g/100 ml @ 18˚ C, ◊ 21.2 g/100 ml @ 25˚ C
Remarks Field for Results	Handbook data

Conclusions

Remarks field	Water solubility approximately 200 g/l at room temperature.

Data Quality

• Reliability	Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks.

References

1. The Merck Index. 9th ed. Rahway, New Jersey:
Merck & Co., Inc., 1976. 1249

Fate in the Environment

Photodegradation

Type **Photodegradation**

Test Substance 1,3,5-Trioxane
CAS Number: 110-88-3

Method

- Guideline: Estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN)[1] which estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical.
- Test Type: Photodegradation Estimate
- GLP: No
- Year: 2000

Result

- Experimental •OH rate constant 6.2×10^{-12} cm^3/molecule-sec (2)
- APOWIN estimated •OH rate constant 10.2×10^{-12} cm^3/molecule-sec

Remarks Field for Results: APOWIN estimated the reaction rate but lists a measured value for this material in the reference materials.[2] Based on the measured rate constant and using the defaults in APOWIN for atmospheric hydroxyl radical concentration, the estimated half-life is approximately 25 hours. Using the APOWIN estimated rate constant the estimated half-life is approximately 12.5 hours.

Conclusions

Remarks field: The atmospheric half-life of 1,2,3-Trioxane in the atmosphere is estimated to be in the range of 25 hours.

Data Quality

- Reliability: Klimisch Code 1. A reliability code of 2 is assigned a result using an accepted method of estimation. Since there is a literature value for the reaction rate of this material with hydroxyl radical, and since it is similar to the APOWIN estimated value the estimate is considered to have a higher reliability and is assigned a code of 1.

References

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Water Stability

Type **Water Stability**

Test Substance 1,3,5-Trioxane
CAS Number: 110-88-3
Purity > 99 % by GC
Source: Fluka (ZAX A 2205/02/GC_731)

Method

- Guideline OECD 111 (1981)
- Test Type Hydrolysis as a Function of pH
- GLP Yes
- Year 2000

Remarks Field for Test Conditions

◊ Duration Six days (preliminary test only)

◊ Analytical Method Direct injection into GC using flame ionization detector.

◊ Buffers

Target pH	Buffer System	Measured pH
4.0	Citric acid/sodium chloride, sodium hydroxide	3.96
7.0	Potassium dihydrogen phosphate/disodium hydrogenphosphate	7.03
9.0	Sodium borate/hydrochloric acid	9.04

◊ Vessels One-liter water-jacketed glass vessels with screw caps.

◊ Replicates One at pH 4 and 7, two at pH 9.

◊ Temperature 49.8 – 50.0 ˚C

◊ Sampling About 12 samplings in six days.

◊ Additional Testing Not conducted since material showed less than ten percent degradation at 50˚ in four days.

Results

- Nominal Target Concentration: 875 mg/l
- Measured Concentrations

Conditions		Measured Concentration (mg/l)		Percent Degradation 6 days
pH	T (deg C)	Initial	Final	
4.06 (±0.05)	49.8 (±0.1)	905	867	4.2
6.98 (±0.05)	50.0 (±0.1)	893	843	5.6
8.84 (±0.05)	49.9 (±0.1)	918	909	1.0
8.85 (±0.05)	50.0 (±0.1)	874	860	1.6

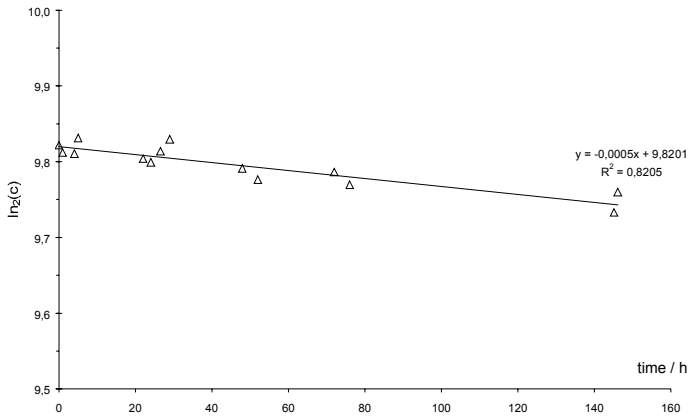
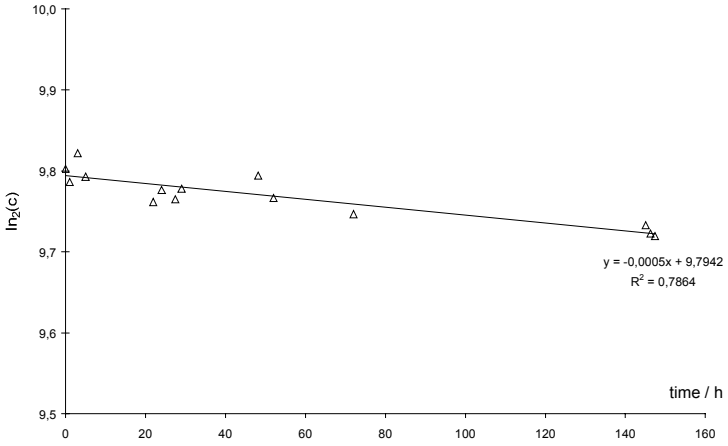
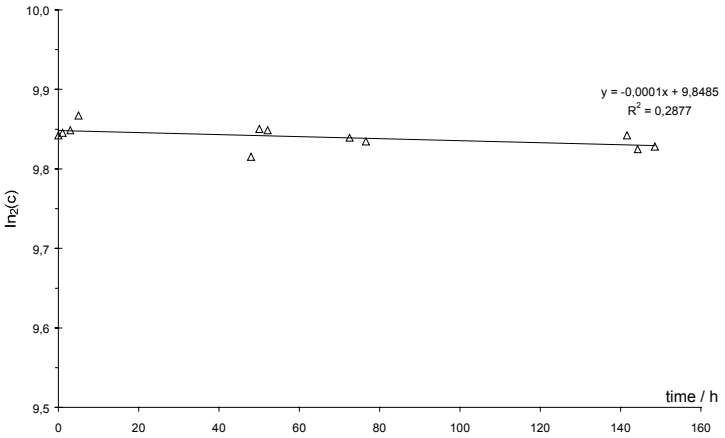
- Percent Degradation: Less than 10% at 50˚ in six days
- Breakdown Products: None

Remarks Field for Results

Sampling times and analysis results for all samples of Trioxane							
pH 4.06		pH 6.98		pH 8.84		pH 8.85	
Time	Conc.	Time	Conc.	Time	Conc.	Time	Conc.
0.0	905	0.0	893	0.0	918	0.0	874
1.0	899	1.1	883	1.0	920	1.0	880
4.1	898	3.0	905	3.0	922	3.0	890
5.0	911	5.0	887	5.0	934	5.0	869
22.0	894	22.0	868	-*	-	-	-
24.0	891	24.1	877	-	-	-	-
26.6	900	27.5	870	48.0	901	48.4	847
29.0	910	29.0	878	50.0	923	50.0	864
47.9	886	48.2	888	52.1	922	52.1	863
52.0	877	52.0	871	72.6	916	72.3	858
72.0	883	72.0	859	76.6	913	76.4	858
76.0	873	145.1	851	141.6	918	141.5	859
145.1	851	146.3	845	144.3	907	144.3	859
146.1	867	147.4	843	148.5	909	148.5	860

* Sampling in the 24-hour time range not conducted for pH 9 determinations

Remarks for Results, continued

Hydrolysis Plots	
pH	Plot
◊ 4.06	
◊ 6.98	
◊ 8.84	 First run only presented, second run is almost identical

Conclusions

Remarks field

- Trioxane is stable at pH 4, 7 or 9 for six days at 50˚ C under the conditions specified in the OECD 111 Guideline.
- Trioxane is estimated to have the following half-life (t1/2) at 25 ˚ C.

Experiment	$t_{½,\ 25°C}$ (days)
pH 4	780
pH 7	840
pH 9	3200
pH 9 (2nd test)	2000

Data Quality

- Reliability

Klimisch Code 1. May be used without restriction.

Reference

Physico-chemical properties of Trioxane (Hydrolysis as a Function of pH.) ZAX Analytik. Study No. 00L00453, BASF AG. Ludwigshafen November 2000.

Other

The hydrolysis-modeling program found in EPIWIN has no valid model for ethers.

Reference for supporting study

1. HYDROWIN modeling program, version 1.67, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Theoretical Distribution (Fugacity)

Type	**Theoretical Distribution (Fugacity)**
Test Substance	1,3,5-Trioxane

Method

- Guideline: Estimated using the MacKay model with standard defaults contained in EPIWIN v 3.05.[1]
- Test Type: Level III Fugacity Model
- GLP: No
- Year: 2000

Result

- Distribution:
  - ◊ Air 4.9 %
  - ◊ Water 53.4 %
  - ◊ Soil 41.6 %
  - ◊ Sediment 0.09 %

Remarks Field for Results:
- ◊ This is the currently accepted model for theoretical distribution estimation.
- ◊ Experimentally determined vapor pressure and water solubility were used to improve the modeling.
- ◊ Calculated K_{oc} is 0.152

Conclusions

Remarks field: This material is expected to environmentally distribute primarily in water and soil

Data Quality

- Reliability: Klimisch Code 2. A reliability code of 2 is assigned a result using an accepted method of estimation.

References

Syracuse Research Corporation, Syracuse, NY (April 2000)

Biodegradation

Type	**Ready Biodegradation**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3

Method

- Guideline: MITI Test
- Test Type: Ready Biodegradation
- GLP: No
- Year: 1981
- Contact Time: 28 days
- Innoculum: Activated sludge from BASF's waste water treatment plant

Result

- Result: % Biodegradation in 28 days 2%

Remarks Field for Results: Not readily biodegradable

Conclusions

Remarks field: Not readily biodegradable

Data Quality

- Reliability: Klimisch Code 2 Study design, conduct and reporting are considered reliable to address the test endpoint although it could not be established that the study was conducted in accord with GLP standards.

References

Weitere Untersuchungen vom Trioxan, 1,3-Dioxepam und Dibutylformal. Respirometrische Tests Uber 28 Tage, TUU/W – K 210 –1130, BASF AG, Ludwigshafen, April 1981.

Other

- This study is supported by an earlier study, sponsored by Celanese, in which Dioxolane was tested for biodegradation using a municipal secondary effluent and measuring oxygen consumption with a manometric respirometer. After 15 days Dioxolane showed 4.8 % of the THOD. [1]
- Other short-term BOD studies conducted by BASF.
- The BIOWIN V4.0 model found in EPIWIN. Two of the three models included predict that Trioxane will not rapidly biodegrade.[2]

References for Supporting Studies

1. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Biodegradation, Inherent

Type	**Inherent Biodegradation**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3

Method

- Guideline: OECD 302B
- Test Type: Inherent Biodegradation, Zahn-Wellens method with aeration
- GLP: No
- Year: 1984
- Contact Time: 23 days
- Innoculum: Activated sludge from BASF's waste water treatment plant

Remarks Field for Test Conditions

◊ Conditions	Trioxane is known to volatilize directly from water. A control was run with aeration only to determine the potential extent of "air stripping"
◊ Acclimation	Waste-water treatment plant receives some Trioxane; thus it is considered pre-acclimated.

Result

- Degradation: Cannot be determined

% Biodegradation in 23 days

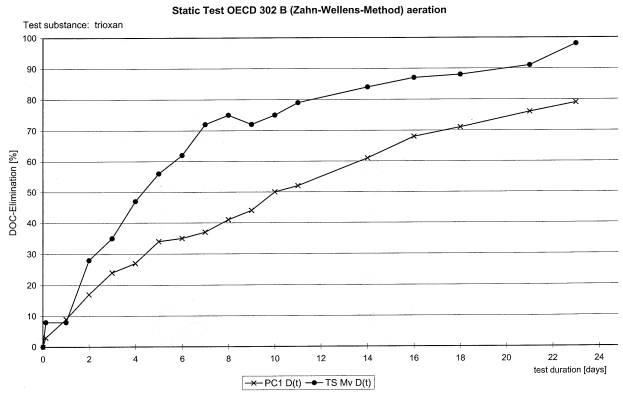
- Results
  - 79% loss of DOC from control due to air stripping.
  - 98 % loss of DOC from inoculated sample due to air stripping and biodegradation
- Breakdown Products: Not identified

Remarks Field for Results

◊ DOC level at Each Time

Day of Test	DOC (mg/l)	
	Aeration only	Aeration plus Bacteria
0	388	386
1	360	378
2	323	297
3	292	267
4	276	219
5	245	183
6	241	156
7	231	120
8	212	108
9	200	118
10	177	109
11	169	93.0
14	134	75.0
16	109	62.0
18	100	60.0
21	83.0	49.0
23	70.0	25.0

◊ DOC Loss Curves



Conclusions

Remarks field

Trioxane was effectively removed from solution by aeration in the presence of a bacterial innoculum; however, the relative contributions of biodegradation and volatilization could not be determined.

Data Quality

- Reliability

Klimisch Code 2 Study design, conduct and reporting are considered reliable to address the test endpoint although it could not be established that the study was conducted in accord with GLP standards.

References Reprint Of A Test Report: Determination of the Ultimate and Inherent Biodegradation and the Elimination from Water of Trioxan in a Batch Test with Activated Sludge BASF Aktiengesellschaft, Ecological Studies D-67056 Ludwigshafen, January 1984

Other This result is supported by the BIOWIN V4.0 model found in EPIWIN that estimates a time of "weeks" for ultimate biodegradation.

References for Supporting Studies Syracuse Research Corporation, Syracuse, NY (April 2000).

Biodegradation, Inherent

Type	**Inherent Biodegradation**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3

Method

- Guideline: Modified OECD 302B
- Test Type: Inherent Biodegradation, Modified Zahn-Wellens method without aeration using Nitrate as oxygen source
- GLP: No
- Year: 1984
- Contact Time: 23 days
- Innoculum: Activated sludge from BASF's waste water treatment plant

Remarks Field for Test Conditions

- ◊ Conditions: Trioxane is known to volatilize directly from water. A previous test showed that loss to air with aeration was significant. This study used a closed system with the addition of 2 g/l Sodium nitrate as an oxygen source. The control was test material with Sodium nitrate without bacteria.
- ◊ Acclimation: The wastewater treatment plant receives some Trioxane; thus, it is considered preacclimated.

Result

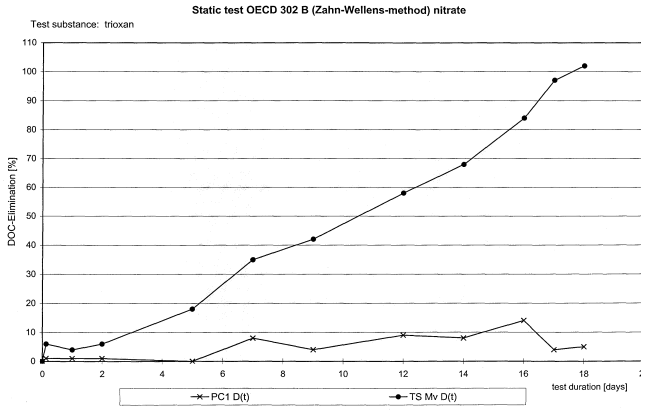
- Degradation
  - ◊ % Biodegradation in 18 days: > 90%
- Results: Inherently biodegradable
- Breakdown Products: Not identified

Remarks Field for Results

◊ DOC level at Each Time

Day of Test	DOC (mg/l)	
	Nitrate only	Nitrate plus Bacteria
0	384	381
1	377	381
2	371	369
5	370	318
7	333	252
9	343	228
12	321	168
14	320	131
16	294	72.0
17	322	28.0
18	314	8.0

◊ DOC Loss Curves



Conclusions

Remarks field

Trioxane was effectively removed from solution under these conditions where volatilization was not possible. It is assumed that essentially complete biodegradation occurred under these conditions.

Data Quality

- Reliability

Klimisch Code 2 Study design, conduct and reporting are considered reliable to address the test endpoint although it could not be established that the study was conducted in accord with GLP standards.

References

Reprint Of A Test Report: Determination of the Inherent and Ultimate Biodegradability of Trioxan in a Modified Batch Test with Activated Sludge Modification: No Aeration but Use of Nitrate as Oxygen Supply BASF Aktiengesellschaft, Ecological Studies D-67056 Ludwigshafen March 1984

Other

This result is supported by the BIOWIN V4.0 model found in EPIWIN that estimates a time of "weeks" for ultimate biodegradation.

References for Supporting Studies

Syracuse Research Corporation, Syracuse, NY (April 2000).

Effects on Environmental Organisms

Acute Toxicity to Fish

Type	**Acute Toxicity to Fish**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3 Purity not specified

Method

- Guideline: The method used closely followed the guideline of DIN 38 412 "Testverfahren mit Wasserorganismen (gruppe L). Allgemeine hinweise zur planung, durchfuehrung und auswertung biologischer Testverfahren (Ll)" und "Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische - Fischtest (ll5)-, June 1982, using a static procedure.
- Test Type: Acute Toxicity to Fish
- GLP: No
- Year: 1988
- Analytical Monitoring: None
- Species/Strain: Golden Orfe (*Leuciscus idus* L., Goldvariante)
- Test Details: Static
- Exposure Period: 96 hours
- Statistical Methods: According to Finney, D.J., Probit Analysis, Cambr. Univ. Press, 3rd Ed., 1971

Remarks Field for Test Conditions

◊	Fish	▪ Length:	Mean 8.1 cm, range 7.2 – 9.3 cm
		▪ Weight	Mean 5.3 g, range 3.6 - 8.3 g
		▪ Loading	5.3 g fish per liter test water
◊	Test Conditions	▪ Type	Static

- Dilution water: Reconstituted freshwater according to DIN 38 412, Part L1, October 1982. Demineralized water resalted with 294.0 mg/l $CaCl_2$x 2 H_20; 123.3 mg/l MgS04 x 7 H20; 63.0 mg/l $NaHC0_3$; 5.5 mg/l KCl
- Water Conditions:
  - Total hardness: 2.5 mmol/l
  - Acid capacity: 0.8 mmol/l
  - Ratio Ca/Mg ions: 4:1
  - Ratio Na/K ions: 10:1
  - pH about 8.0
- Photoperiod: 16 hours light and 8 hours dark
- Water Temp: 20- 21 deg C at all test times

◊ Solvent: Test water
◊ Vessel: All-glass aquaria, 30 X 22 X 24 cm, containing 10 liters
◊ Fish per group: Ten, one replicate per concentration
◊ Fish per vessel: Ten
◊ Exposure period: 96 hours
◊ Observation times: 1,4, 24, 48, 72 and 96 hours
◊ Solution pH range: 7.4 -7.8 at all concentrations and at 1, 24, 28, 72 and 96 hours
◊ Dissolved oxygen: Above 7.8 mg/l for all solutions at 0 and 48 hours

Results

- Nominal Concentrations: 0, 1000, 2150, 4640 and 10000 mg/l
- Units: mg./l.
- LC_{50}: 4030 (96, 72 and 48 hour), 5340 (24 hour)
- LC_0: 2150 (24 to 96 hours)

Remarks Field for Results

◊ Mortality

Nominal Conc	Number Dead Fish After						
(mg/ml)	0 hr	1 hr	4 hr	24 hr	48 hr	72 hr	96 hr
1,000	10	0	0	0	0	0	0
2,150	10	0	0	0	0	0	0
4,640	10	2	3	3	7	7	7
10,000	10	10	10	10	10	10	10

◊ Biological Observations: Restricted to mortality

◊ Control Mortality: Zero

Conclusions

Remarks field

◊ The LC_{50} was found to be 4030 mg/l at 96, 72 and 48 hours; it was 5340 at 24 hours.

◊ The LC_0 was found to be 2150 mg/l at all time intervals.

◊ The NOEC was 2150 mg/l. The lowest concentration causing 100% mortality was 10000 mg/ml.

◊ The study closely followed the OECD 203 guideline with the exception of the recommended species of fish.

Data Quality

- Reliability: Klimisch Code 2 . Study design, conduct and reporting are considered reliable to address the test endpoint although it could not be established that the study was conducted in accord with GLP standards. Study design and reporting meets current EPA/OECD guidelines with minor exceptions

References

Bericht uber die Prufung der akuten Toxizitit an der Goldorfe (Leuciscus idus L., Goldvariante); englische Fassung Prufung zur Einstufung in Wassergefahrdungsklasse. Substance 87/525, 1,3,5-Trioxane. BASF Department of Toxicology July 1988.

Other

- ◊ This study is supported by an earlier study, sponsored by Celanese, in which Sheepshead minnows (Cyprinodon variegates, 5 per group) were exposed to Trioxane at concentrations of 10000, 20000 and 30000 mg/l. In this study, the 96 hour-LC_{50} was reported to be 16350 mg/l. and the 96-hour LC0 was 10000 mg/l [1]
- ◊ The EPA ECOSAR Modeling Program found in EPIWIN, estimates the 96-hour LC_{50} for fish to be 17000 mg/l.[2]

References for supporting studies

1. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Acute Toxicity to Aquatic Invertebrates

Type	**Acute Toxicity to Aquatic Invertebrates**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3 Purity not specified

Method

- Guideline: OECD 202 (1984)
- Test Type: Daphnia, acute immobilization
- GLP: No
- Year: 1989
- Analytical Procedures: None
- Species/Strain: *Daphnia magna* STRAUS
- Test Details: Static
- Statistical Methods: None necessary

Remarks Field for Test Conditions

◊ Age at study initiation	Four to 24 hours
◊ Test conditions	As specified in OECD 202.
◊ Solvent	Test water
◊ Vessel	50 ml glass beaker with 20 ml solution
◊ Daphnids per group	Twenty
◊ Daphnids per vessel	Five
◊ Exposure period	48 hours
◊ Observation times	3, 6. 24, 48 hours
◊ Solution pH range	7.72 to 7.80 at all concentrations at 0 and 48 hours
◊ Dissolved oxygen	Above 8 mg/l for all solutions at 0 and 48 hours

Results

- Nominal Concentrations: 0, 0.1, 1, 10, 100 and 1000 mg/l
- Units: mg./l.
- EC_{50}: > 1000 at 24 and 48 hours
- EC_0: > 1000 at 24 and 48 hours

Remarks Field for Results	◊ Immobilization	o No animal was immobilized at any observation time. o No other adverse effects were reported o Controls were normal

Conclusions

Remarks field

The EC_{50} (48 hour) and EC_0 (48 hour) were greater than 1000 mg/l under these conditions. The study closely followed the OECD 202 guideline.

Data Quality

- Reliability

Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although it could not be established that the study was conducted in accord with GLP standards. Study design and reporting meets current EPA/OECD guidelines with minor exceptions.

References

Fraunhofer-Institute fur Umweltchemie und Okotoxikologie, Daphnia, Acute Immobilisation, Test-Substanz: 1,3,5-Trioxane. 27.4.1989 IUCT-Nr.: BALU1

Other

This study is supported by an earlier study, sponsored by Celanese, in which Trioxane was tested at 5000, 10000, 15000, 20000 and 30000 mg/l. In this study the 48 hour-EC_{50} was reported to be 15200 mg/l. [1]

The EPA ECOSAR Modeling Program found in EPIWIN, estimates the 48-hour LC_{50} for daphnia to be 15000 mg/l.[2]

References for supporting studies

2. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
3. ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Toxicity to Aquatic Plants

Type	**Toxicity to Aquatic Plants**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3 Purity not specified

Method

- Guideline: OECD 201
- Test Type: Algae Growth Inhibition
- GLP: No
- Year: 1990
- Species/Strain: *Scenedesmus subspicatus* strain SAG 86.81
- Element Basis: Cell growth
- Exposure Period: 72 hours
- Analytical Monitoring: No
- Statistical Methods: None necessary due to lack of significant inhibition

Remarks Field for Test Conditions

◊ Test Temperature Range	Stated as 20˚ C
◊ Growth Medium Chemistry	OECD specified medium
◊ Exposure Vessel	Erlenmeyer flask (250 ml) congaing 100 ml media
◊ Water Chemistry	pH remained between 7.9 and 9.7 with all test concentration similar to control.
◊ Light Level and Quality	Not specified in report
◊ Test Design	o Replicates: Four replicate determinations o Concentrations: 0, 3.9, 7.8, 15.625, 31.25, 62.5, 125, 250 and 500 mg/l
◊ Method of calculating mean	Arithmetic
◊ Exposure period	24, 48 and 72 hours

◊ Deviations from OECD Guideline: The study report does not specifically state that it complied with the OECD guideline; however, except for the temperature being one degree lower than recommended by the guideline, all other criteria were met.

Results

- Nominal Concentrations: 0, 3.9, 7.8, 15.625, 31.25, 62.5, 125, 250 and 500 mg/l
- Units: mg./l.
- EC_{20}: > 500
- EC_{50}: > 500
- EC_{90}: > 500
- NOEC: 500

Remarks Field for Results

◊ Biological Observations: None noted

◊ Cell Density in Each Flask at Each Time Point

Conc (mg/l)	Cell Growth (Fluoresence Units)		
	24 hrs	48 hrs	72 hrs
Control	262	812	2103
3.9	259	909	2368
7.8	253	825	2194
15.625	263	810	2157
31.25	246	792	2100
62.5	228	716	2023
125	227	705	1988
250	203	674	1766
500	212	675	1769

Conclusions

Remarks field: The EC_{20} (48 hour) and EC_{50} and EC_{90} (72 hour) were greater than 500 mg/l under these conditions.

Data Quality

Reliability

Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although it could not be established that the study was conducted in accord with GLP standards. Study design and reporting meets current OECD guidelines with minor exceptions.

References

Determination of the inhibitory effect of 1,3,5-Trioxane on the cell multiplication of algae. BASF AG, Ludwigshafen/RheinGermany. Project Number 2/w569/89/t0, 2/w569/89/t24, 2/w569/89/t48 and 2/w569/89/t730. Translation of a report originally dated 23 Feb 1990.

Other

This study is supported by an earlier study, sponsored by Celanese, in which Trioxane was tested for growth inhibition of *Selenastrum capricornutum.* In this study, algae growth was measured out to 14 days of exposure at Trioxane concentrations of 1000, 5000 or 10000 mg/l. Significant inhibition was seen only at 10000 mg/l and 5000 mg/l was determined to be the NOEC. [1]

This study is also supported by the EPA ECOSAR Modeling Program found in EPIWIN[2], which estimates the 96-hour EC_{50} for green algae to be 8245 mg/l.

References for supporting studies

1. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Acute Health Effects

Acute Oral Toxicity

Type	**Acute Oral Toxicity**
Test Substance	Trioxane CAS Number: 110-88-3 Clear colorless crystalline material

Method

- Guideline: Federal Hazardous Substance Labeling Act provisional guidelines FR 8/12/1961
- GLP: No
- Year: 1962
- Species: Rat
- Strain: Albino, unspecified strain
- Route of administration: Oral Gavage
- Doses: 2520, 5000 and 10000 mg/kg
- Sex: Male
- Number of Animals/group: Five
- Vehicle: Water

Remarks Field for Test Conditions

◊ Age at Study Initiation	Unknown, weight between 229 and 281 g
◊ Doses	2520, 5000 and 10000 mg/kg
◊ Volume administered	1 to 12 ml aqueous solution
◊ Post-dose observation period	14 Days
◊ Other	The high does was split into two administrations in a single 8-hour period. Animals were not fasted.

Results

- LD_{50} 8190 mg/kg (95% confidence limits of 6210 to 10080 mg/kg)
- Number of deaths at each dose level

Dose	Mortality
2520 mg/kg	0/5
5000 mg/kg	0/5
10000 mg/kg	4/5

Remarks Field for Results

◊ Time of death: Not stated

◊ Clinical Signs: Not stated

◊ Body Weights

Dose (mg/kg)	Initial weight	Weight gain	Control gain
2520	263	+24	+60
5000	249	+39	+60
10000	248	+23	+60

◊ Necropsy Findings: None

◊ Target Organs: None identified

Conclusions

Remarks field: Study documentation is good for the time. Results are consistent with other data for the material, including a literature study. The study appears to have been well conducted.

Data Quality

- Reliability: Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with GLP standards.

References

Range Finding Tests on Trioxane for Celanese Corporation of America. Industrial Hygiene Foundation of America, Inc, Mellon Institute, October November 1962.

Other

This study is supported by a study in the literature that reports the acute oral LD_{50} in the rat to be 8,500 mg/kg[1]

References for supporting studies

1. Czajkowska, T, Krysiak, B and Popiânska, E. Experimental studies of toxic effects of 1,3,5-trioxane and 1,3-dioxolane. I. Acute toxic effect. Med Pr; Vol 38, 1987, P184-90.

Acute Inhalation Toxicity

Type	**Acute Inhalation Toxicity**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3 C-235 Lot 41115

Method

- Guideline: None specified
- GLP: Yes
- Year: 1986
- Species: Rat
- Strain: Charles River CD®
- Route of administration: Whole-body inhalation as vapor
- Doses: 8,370 and 10,643 ppm (maximum attainable concentration at high dose)
- Sex: Males and Females
- Exposure Period: Four hours
- Number of Animals/group: Five animals of each sex per dose level
- Vehicle: Air

Remarks Field for Test Conditions	◊	Age at Study Initiation	Males: 9 weeks Females: 12 weeks
	◊	Doses	Initial dose was 8,370 ppm (measured), as mortality was not observed, a second group was exposed at the maximum attainable vapor concentration, which was measured at 10,643 ppm. Particle size distribution analysis showed no particles were present, thus it is assumed that this was a vapor exposure.
	◊	Post-dose observation period	14 Days

Results

- LC_{50} >10,643 ppm

- Number of deaths at each dose level No deaths reported

Remarks Field for Results

Clinical Signs

8,370 ppm

Sign	Onset	Duration	# Animals
Lacrimation	4 hours	14 days	Few
Shallow breathing	4 hours	< 24 hours	6/10
Irregular breathing	4 hours	2 days	9/10
Reduced activity	45 minutes	< 24 hours	Most
Nasal discharge	4 hours	14 days	About half

Clinical Signs

10,643 ppm

Sign	Onset	Duration	# Animals
Lacrimation	15 minutes	13 days	many
Shallow breathing	4 hours	< 24 hours	half
Irregular breathing	30 minutes	5 days	All
Reduced activity	15 minutes	< 24 hours	All
Nasal discharge	4 hours	14 days	Few to most

Mean Body Weights

8,370 ppm

10,643 ppm

Day	0	2	3	5	8	15
Males	338	310	319	325	342	364
Females	230	210	218	221	233	239
Males	329	293	292	310	330	352
Females	226	210	216	216	224	235

Necropsy Findings

8370 ppm

Encrusted ear	1/10
Dilated kidney pelvis	1/10
Discolored lymph node	1/10

Necropsy Findings 10,643 ppm	Encrusted ear	2/10
	Dilated kidney pelvis	1/10
	Calculi in kidney pelvis	1/10
	Discolored lymph node	1/10
	Ureter distended	1/10
	Urinary bladder thickened	1/10
	Urinary bladder calculi	1/10

Other: Necropsy findings considered unremarkable. No target organs were identified at necropsy. Response of males and females was similar. There was a compound-related reduction in body weight following exposure. Animals gained body weight normally during the second week of the observation period

Conclusions

Remarks field: All test animals survived until termination. There was a slight dimunition in body weight for each sex in each group following exposure; however, body weights during the second week were unremarkable. Other signs of treatment included increased secretory response, respiratory distress, and general signs of poor condition. Gross postmortem findings were considered unremarkable.

Data Quality

- Reliability: Klimisch Code 1 Although not a guideline study, the study was substantially similar, well documented, conducted under GLPs and measured concentrations of test material were used.

References

An Acute Inhalation Toxicity Study of Trioxane (C-235) in the Rat. Bio/dynamics Inc. Project 85-7832, submitted to Celanese Corporation, Feb 27 1986.

Other

This study is supported by a study in the literature that reports the acute inhalation LC_{50} to be greater than 26000 mg/m^3 (>6500 ppm).[1]

References for supporting data

1. Czajkowska, T, Krysiak, B and Popiânska, E. Experimental studies of toxic effects of 1,3,5-trioxane and 1,3-dioxolane. I. Acute toxic effect. Med Pr; Vol 38, 1987, P184-90.

Acute Dermal Toxicity

Type	**Acute Dermal Toxicity**
Test Substance	Trioxane CAS Number: 110-88-3 Clear colorless crystalline material

Method

• Guideline	Federal Hazardous Substance Labeling Act provisional guidelines FR 8/12/1961
• GLP	No
• Year	1962
• Species	Rabbit
• Strain	Albino, unspecified strain
• Route of administration	Dermal administration
• Doses	3,980 mg/kg
• Sex	Male
• Exposure Period	Twenty-four hours
• Number of Animals/group	Four
• Vehicle	None

Remarks Field for Test Conditions	◊	Age at Study Initiation	Unknown
	◊	Doses	Single dose level of 3,980 mg/kg.
	◊	Post-dose observation period	14 Days
	◊	Other	Two animals had skin abraded prior to exposure

Results

• LD_{50}	>3,980 mg/kg
• Number of deaths at each dose level	No deaths reported

Remarks Field for Results

Clinical Signs 3,980 mg/kg: The only reported clinical sign was very slight to moderate erythema but no appreciable edema of the skin after removal of the wrap. This disappeared by study termination

Mean Body Weights

Sex	Day	
	0	14
Males	2520 g	2570 g

Necropsy Findings: None

Conclusions

Remarks field: All test animals survived until termination. Two of the four rabbits showed a reduction in body weight gain as compared to controls. Gross postmortem findings were unremarkable. The material produced slight to moderate skin irritation.

Data Quality

- Reliability: Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with GLP standards.

References

Range Finding Tests on Trioxane for Celanese Corporation of America. Industrial Hygiene Foundation of America, Inc, Mellon Institute, October-November 1962.

Other

This study is supported by a study in the literature that reports the acute dermal LD_{50} in rabbits to be greater than 15000 g/kg (1).

References for supporting data

1. Czajkowska, T, Krysiak, B and Popiânska, E. Experimental studies of toxic effects of 1,3,5-trioxane and 1,3-dioxolane. I. Acute toxic effect. Med Pr; Vol 38, 1987, P184-90.

Repeated Dose Toxicity, 28-Day Oral

Type	**Repeated Dose Toxicity, 28-Day Oral**
Test Substance	Trioxane CAS Number: 110-88-3 99.1% purity (0.8% water, 0.1% Triethanolamine)
Method	
• Guideline	OECD 407 Repeat Dose Oral Toxicity (1981)
• GLP	Yes
• Year	1990
• Species	Rat
• Strain	Wistar [Hoe: WISKf(SPF71)] Source: Hoechst AG, Kastengrund
• Route of administration	Oral gavage
• Duration of Test	29 days
• Doses	0, 40, 200, or 1000 mg/kg/day
• Sex	Male and Female
• Exposure Period	Once daily
• Frequency of Treatment	◊ Seven days a week ◊ Twenty-eight doses in twenty-nine days
• Number of Animals/group	Five of each sex
• Vehicle	Deionized water
• Control Group and Treatment	Five animals of each sex dosed with vehicle (water)
• Post-Exposure Observation Period	Twenty-four hours after last dose.
• Statistical Methods	Referenced established methods

Remarks Field for Test Conditions	◊ Age at study initiation	Six weeks
	◊ Number of animals per Sex per dose	Five of each sex per concentration
	◊ Satellite groups	None
	◊ Housing	Housed 5 to a group on soft wood granules
	◊ Test Material Preparation	Trioxane was prepared fresh each day of dosing and was not analyzed for concentration or stability. It is known to be relatively stable in water solution.
	◊ Rationale for dose selection	The high-dose of 1000 mg/kg day is the maximum dose level recommended in the OECD Guideline. The fivefold spacing was selected to assure that a NOEL would be established.
	◊ Clinical observations performed and frequency	▪ Mortality and gross signs: Twice daily except once daily on weekends and holidays. ▪ Body weights were measured twice a week ▪ Possible neurological disturbances were evaluated weekly ▪ Weekly examinations were conducted for the following: eyes for signs of opacity, the mucus membranes of the mouth for damage, and the teeth for unusual growth.
	◊ Terminal observation	▪ Blood taken for hematology and clinical chemistry (Na, K, PO4, uric acid, creatinin, glucose, BUN, Ca, Cl, SGOT, SGPT, AP, GGT, total protein, albumin, globulin (calculated) A/G ratio). ▪ Serum electrophoresis. ▪ Urinalysis for a 16 hour sample ▪ Gross postmortem examination including external surfaces, all orifices, eyes, teeth, and inner organs of all animals. ▪ Organ weights for heart, lungs, liver, kidneys, spleen, testes and adrenals. ▪ Microscopic examination for several tissues.
	◊ Histopathology	The following tissues were examined from all animals: Heart, lungs, liver, kidneys, spleen, testes and adrenals, thymus, jejunum, colon, bone marrow and stomach.
	◊ Differences from current guideline	The current OECD 407 guideline (adopted in 1995) recommends additional tissues for gross and histopathological examination.

Results

- NOAEL 200 mg/kg /day
- LOEAL 1000 mg/kg/day

- Mortality All animals survived the duration of the study.

Toxic Responses

Dose	Toxic Responses
40 mg/kg	None
200 mg/kg	None
1000 mg/kg	▪ Males: Significant decrease in leucocyte count, increase in gamma-glutamyl-transpeptidase. No changes in body or organ weight nor any significant histopathological findings. The study director did not consider the finding of a single animal reported to show testicular atrophy significant. ▪ Females: Significant decrease in leucocyte count, increase in gamma-glutamyl-transpeptidase, increase in SGPT and SGOT, decrease in serum protein and glucose levels. No changes in body or organ weight nor any histopathological findings

Remarks Field for Results

◊ Body Weights (Males)

Dose (mg/kg)	Initial weight	Final weight	Weight gain
0	124±7	282±31	158±25
40	122±7	282±15	160±13
200	121±3	281±15	161±05
1000	122±5	257±22	135±18

◊ Body Weights (Females)

Dose (mg/kg)	Initial weight	Final weight	Weight gain
0	112±4	195±15	83±10
40	112±2	195±10	83±12
200	110±6	194±14	83±16
1000	113±4	188±07	75±04

◊ Food/water

	Food Consumption (g/100g body wt/day)		Water Consumption (g/100g bodywt/16 hr)	
Dose	Males	Females	Males	Females
0	10.0	9.7	13.1	12.4
40	10.2	10.1	12.5	12.3
200	10.4	10.1	12.6	14.8
1000	9.7	9.9	11.9	10.3

◊ Clinical Signs

No clinical signs of toxicity were observed.

◊ Hematologic Results (Selected Values)

	Leukocytes (10^9/L)	
Dose (mg/kg)	Males	Females
0	6.1±0.6	4.3±0.8
40	6.3±1.4	5.5±1.2
200	4.8±0.1	4.7±2.2
1000	3.6±0.6*	2.6±0.5*

◊ Clinical Chemistry

	Selected Clinical Chemistry Values - Males				
Dose (mg/kg)	GGT (U/L)	SGOT (U/L)	SGPT (U/L)	Protein (g/L)	Glucose (mmol/L)
0	0±0	77±11	43±12	57±4	10.8±6.2
40	0±0	68±06	36±03	56±3	9.6±0.8
200	1±1	72±09	43±03	57±2	10.5±0.8
1000	1±1	75±12	58±14	56±1	10.4±1.1

	Selected Clinical Chemistry Values - Females				
Dose (mg/kg)	GGT (U/L)	SGOT (U/L)	SGPT (U/L)	Protein (g/L)	Glucose (mmol/L)
0	1±1	64±4	31±3	59±4	16.6±3.4
40	0±0	71±16	31±3	57±3	11.9±1.3
200	0±1	61±9	33±5	56±2	12.0±5.9
1000	1±1	84±9*	45±2*	53±2*	7.5±0.9*

Other chemistry parameters showed significant changes from controls but were considered not compound-related as they were within the range of historical controls for this strain.

◊ Necropsy Findings

None reported

◊ Organ Weight

The absolute spleen weights of high-dose males were significantly reduced compared to controls, relative spleen weighs of males were not significantly affected. In females, the mid-dose mean spleen weight was significantly higher than control but this was disregarded due to lack of dose response.

	Selected Final Organ Weights, males (grams)			
Dose (mg/kg)	Spleen	Kidney	Testes	Liver
0	0.61±0.12	1.85±0.19	2.99±0.33	11.61±1.50
40	0.60±0.06	1.89±0.10	2.95±0.28	11.05±1.26
200	0.63±0.07	1.85±0.12	3.19±0.18	11.30±0.94
1000	0.47±0.06*	1.65±0.25	2.48±1.02	11.40±1.66

	Selected Final Organ Weights, females (grams)			
Dose (mg/kg)	Spleen	Kidney	Liver	
0	0.42±0.03	1.45±0.14	8.44±0.83	
40	0.47±0.03	1.35±0.07	7.84±0.62	
200	0.52±0.06*	1.45±0.09	8.47±0.69	
1000	0.39±0.05	1.29±0.09	7.74±0.56	

◊ Histopath: The examining pathologist considered no finding significant. Few findings were reported and treated animals were similar to controls. Of interest for this material is the testis, in which one high-dose male (of five) was reported to show testicular atrophy.

◊ Target Organs: Blood leucocytes, liver

◊ Other: Serum electrophoresis results were similar for treated and control groups.

Conclusions

Remarks field

The NOEL was determined to be 200 mg/kg/day. Adverse clinical effects were not observed at any dose level. Body weight gain was unaffected; food and water consumption were similar for all groups. Leukocyte levels were significantly depressed at the high-dose level. Clinical chemistry parameters indicative of hepatic damage were elevated in high-dose females but no corresponding histopathologic effects were observed.

This is a well-documented guideline study conducted under GLPs

Data Quality

- Reliability: Klimisch Code 1. Reliable without restriction, study meets GLP standards and/or most requirements.

References

Trioxane. Subakute orale Toxizitat (28 Application in 29 Tagen) an SPF-Wistar-Ratten. Hoechst AG Central Toxicology Report number 90.0513 22 May 1990.

* = $p < 0.05$

Other

This study is supported by a gavage study of duration four or seven months, depending on the dose level, conducted using male rats. In this study, groups of 8-10 rats were administered Trioxane as a water solution, 5 days a week. The high dose was 850 mg/kg and the duration of dosing was 4 months. Doses of 213 or 106 mg/kg were administered for 7 months. Body weight gain, as compared to controls, was slightly reduced at 850 and at 213 mg/kg and slightly increased at 106 mg/kg. At the end of the exposure period, blood was taken and evaluated for several hematology and clinical chemistry parameters. Heart, liver, lung, kidney, adrenals and spleen were weighed at necropsy and examined microscopically. No specific organ effects were reported and it was concluded that the mortality rate did not show unexpectedly high mortality or other toxicity with longer-term exposure. Examination of the limited data presented suggest that 213 mg/kg was near the NOAEL for Trioxane after 7-months of administration. It could not be determined if the same parameters were examined in high-dose animals, which were sacrificed after only 4 months of exposure, or if a concurrent control group was sacrificed with the high-dose group. It is not known if there were any effects on spleen or WBC's as the information available for this study lacks sufficient documentation for through evaluation and comparison with the repeated-dose studies.

Reference for supporting study	Experimental studies of the toxic effects of 1,3,5-trioxane and 1,3-dioxolane. II. Cumulation of toxic effect. Czajkowska T; Krysiak B Med Pr; VOL 38, 1987, P244-9

Repeated Dose Toxicity, Two-Week Inhalation

Type	**Repeated Dose Toxicity, Two-Week Inhalation**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3 White crystalline solid, C-235
Method	
• Guideline	None Specified
• GLP	No
• Year	1983
• Species	Rat
• Strain	CD (Sprague-Dawley derived) Charles River, Wilmington MA
• Route of administration	Whole body inhalation of vapor
• Duration of Test	12 days
• Doses	0, 103, 984 or 4940 ppm (Mean, measured concentrations)
• Sex	Male and Female
• Exposure Period	Six hours per day
• Frequency of Treatment	Five days a week Ten exposures in twelve days
• Number of Animals/group	Five of each sex
• Control Group and Treatment	Five animals of each sex exposed only to air under the same chamber conditions
• Post-Exposure Observation Period	None, animals sacrificed immediately after last exposure

- Statistical Methods
  - ◊ Body weight data, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of the treated groups were compared to control at each time interval.
  - ◊ Statistical evaluation of equality of means was made by the appropriate one-way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one-way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.
  - ◊ A statistical test for trend in the dose levels was also performed. In the parametric case, (i.e. equal variance) standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case, Jonckheere's test for monotonic trend was used.
  - ◊ The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

Remarks Field for Test Conditions

◊ Age at study initiation	▪ Males: 41 days ▪ Females: 58 days
◊ Number of animals per Sex per dose	Five of each sex per concentration
◊ Satellite groups	None
◊ Housing	Individually housed in stainless steel cages
◊ Clinical observations performed and frequency	▪ Mortality and gross signs: Twice daily ▪ Abnormal signs: Daily ▪ Detailed physical examination: Twice weekly
◊ Terminal observations	▪ Blood taken for hematology and clinical chemistry (sgpt, bun, glucose, total protein, albumin, globulin (calculated) A/G ratio). ▪ Complete gross postmortem examination including external surfaces, all orifices, the cranial cavity, carcass, the external surface of the brain and spinal cord, the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissues and organs were examined for all animals.

◊ Histopathology — The following tissues were examined from all control and high-exposure animals only. Bone marrow smear, right kidney, liver, lungs, peribronchial lymph nodes, nasal turbinates and thymus.

Results

- NOAEL
  - ◊ Males: Not determined (< 103 ppm)
  - ◊ Females: 103 ppm
- LOEAL
  - ◊ Males 103 ppm
  - ◊ Females: 984 ppm
- Mortality

All animals survived the duration of the study.

- Toxic Responses

Dose	Toxic Response
◊ 103 ppm	A decrease in mean absolute and relative spleen weights was observed for males only. All other findings occurred sporadically and were not considered to be related to exposure
◊ 984 ppm	A decrease in mean absolute and relative spleen weights was observed for males and females. All other findings occurred sporadically and were not considered to be related to exposure
◊ 4940 ppm	Exposure-related effects were noted in each sex of this group. These included increased secretory responses, reduced righting reflex and grip strength, persistent pupillary constriction and decreased mean body weights throughout most of the study. In addition, hemoglobin, hematocrit, erythrocyte counts and lymphocyte counts were elevated, while total leukocytes and segmented neutrophils were decreased. Slight to significant increases in mean serum glutamic pyruvic transaminase, total protein and albumin values, and a decrease in glucose values were also noted. In the absence of supportive microscopic findings in the liver and kidneys of these animals, the significance of the clinical pathology is unclear. Mean absolute and relative spleen weights were decreased for the high-dose animals. Increased mean relative weights for all other weighed organs were associated with significantly decreased mean terminal body weights for this group. The mucosa of the anterior nasal cavity from the high-exposure males and females showed squamous metaplasia with necrosis and desquamation. Acute rhinitis with neutrophil exudation into the nasal cavity was a concomitant change.

Remarks Field for Results ◊ Body Weights

	Test Day and Time					
Males	Pretest	1 (AM)	1 (PM)	5 (AM)	5 (PM)	12 (AM)
Control	162	202	189	224	214	276
103 ppm	163	203	190	226	215	278
984 ppm	162	202	187	223	212	268
4940 ppm	163	207	189	202**	193**	239**
Females						
Control	183	201	191	198	193	228
103 ppm	182	197	184	205	195	219
984 ppm	183	199	185	203	192	213
4940 ppm	183	199	183	189	179	200**

◊ Clinical Signs

Clinical Signs		Day (# Animals/10)			
		2	5	9	12
0 ppm	Lacrimation	0	0	0	1
	Mucoid nasal discharge	0	0	0	1
	Swollen conjunctiva	0	0	0	4
103 ppm	Lacrimation	2	2	1	5
	Mucoid nasal discharge	8	8	1	0
	Dry rales	2	0	0	1
	Swollen conjunctiva	0	0	0	4
984 ppm	Lacrimation	9	5	0	7
	Mucoid nasal discharge	10	9	3	1
	Red nasal discharge	0	0	0	1
	Salivation	1	1	0	0
	Dry rales	1	0	0	0
	Swollen conjunctiva	0	0	0	1
4940 ppm	Lacrimation	10	1	2	6
	Mucoid nasal discharge	9	10	7	6
	Red nasal discharge	0	0	0	1
	Dry rales	0	0	0	4
	Moist rales	0	0	2	1
	Reduced righting reflex	10	6	10	9
	Reduced grip	9	1	9	5
	Persistent pupil constriction	8	0	8	1
	Swollen conjunctiva	0	0	0	3
	Yellow fur	0	0	4	7
	Negative toe pinch reflex	1	0	0	0
	Yellow ano-genital fur	1	1	2	1

◊ Hematology

PPM	HGB	HCT	RBC	Platelets	MCV	MCH	MCHC	Clot T	WBC
Males	g/dl	%	10^6/mm	10^5/mm	μ^3/cell	µµg	g/dl	min	103/mm
0	13.6	40	6.20	15.22	65	22	33.9	1.4	13.9
103	14.0	41	6.28	13.43	66	22.3	33.9	1.6	13.8
984	13.9	41	6.11	14.16	67	22.7	34.1	1.4	13.3
4940	15.2**	45**	6.93**	15.14	64	22	34.2	1.7	6.2**
Females									
0	14.1	42	6.45	10.77	65	21.9	33.7	1.6	11.0
103	14.5	43	6.76	13.24	64	21.5	33.3	1.5	11.0
984	14.8	44	6.79	13.26	65	21.8	33.8	1.7	10.8
4940	15.4**	46**	7.13*	11.46	64	21.7	33.7	1.5	8.5

◊ Necropsy findings: Gross postmortem examinations revealed no differences among groups which were considered to represent an effect of exposure to the test substance

◊ Organ weights: Mean absolute and relative spleen weights were decreased for the 103 ppm-group males and the mid and high-dose males and females; differences were indicative of an exposure-response relationship. Increased mean relative weights for all other weighed organs (brain, heart, kidneys, liver, testes/ovaries) were noted for the high-dose males and/or females; however, these differences were associated with the significantly decreased mean terminal body weights exhibited by these animals.

◊ Histopath-ology: Significant changes in the mucosa of the anterior nasal cavity were noted in the high-dose males and females. The columnar ciliated epithelium was replaced by a less specialized squamous epithelium. The metaplasia was focal, and the epithelium showed various degrees of degeneration, necrosis and desquamation. Concomitant with this change was an acute inflammation of the nasal mucosa characterized by collections of neutrophils in the lamina propria and mucosa. Exudation of neutrophils produced an accumulation of purulent exudate in the nasal cavity. In a few cases, a small layer of packed neutrophils was adherent to the mucosa. Proteinaceous material, usually with enmeshed neutrophils, was seen predominantly in the high-dose males. Microscopic changes in other organs occurred sporadically in both the control and high-exposure animals and did not indicate any exposure related effects.

Findings in the Nasal Turbinate (Nl section)	Exposure Concentration (ppm)			
	0	103	984	4940
Acute Rhinitis	0/10	3/10	6/10	7/10
Mucosa:				
-Squamous Metaplasia	0/10	0/10	8/10	10/10
-Erosion	0/10	0/10	7/10	10/10
-Hyperplasia	0/10	0/10	2/10	0/10

Conclusions

Remarks field: Two-weeks of inhalation exposures at concentrations of 984 ppm and above produced a variety of toxic sequelae including a decrease in weight gain, splenic atrophy and squamous metaplasia in the mucosa of the anterior nasal passage. The LOAEL was 103 ppm for males showing a decrease in mean absolute and relative spleen weights. 103 ppm was considered a NOAEL for females.

Study documentation is good. Results are consistent with other data in the literature and the study appears to have been well conducted.

Data Quality

- Reliability: Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with GLP standards.

References Bio/Dynamics Inc. A Two-Week Inhalation Toxicity Study of C-235 in the Rat. Project Number 82-7572 1983 Submitted to Celanese Corporation.

Other This study is supported by a 12-month inhalation study in the literature where concentrations of 50 500 and 2500 mg/m^3 were found to be toxic to rats or guinea pigs, and a NOEL concentration of 50 mg/m^3 was reported. (50 mg/m^3 is about 12 ppm) The 500 mg/m^3 groups and higher showed adverse pathological changes of the kidneys and respiratory epithelium. Few details are available. Considering the extended length of this study and that the next higher dose level was 500 mg/m^3 (about 120 ppm), which was apparently a LOAEL; the 103 ppm NOAEL/LOAEL from the 14-day inhalation study is in accord with the longer study. It is not known if there were any specific adverse effects on spleen or WBC's in this long-term study since the available report lacks sufficient documentation for adequate evaluation and comparison with the repeated-dose study.

References for supporting studies

1. Indulski et al. MAC Values for trioxane and dioxolane at the work place proposed on the basis of animal studies. Fourteenth International Congress on Occupational Health in the Chemical Industry (MEDICHEM), pp 548-556 (1986). As summarized in Toxikologische Bewertung. Heidelberg, Berufsgenossenschaft der chemischen Industrie *Trioxane* Nr. 185 (1992)

** = $p < 0.01$

Genetic Toxicology *in vitro*

Reverse mutation assay, Ames Test

Type **Reverse mutation assay - *S. typhimurium***

Test Substance

1,3,5-Trioxane
CAS Number: 110-88-3
Substance-Nr.:88/164

Method

- Guideline: None specified
- GLP: No
- Year: 1988
- Species:
  - ◊ S. typhimurium: TA1535, TA100, TA 1537, TA98,
  - ◊ Metabolic activation. Tested with and without
  - ◊ Rat liver S-9
  - ◊ 0.5 ml S-9 per 100 ml agar plate when used
  - ◊ Triple plate
  - ◊ Independent repeat using pre-incubation method for repeat
- Concentrations tested:

Concentrations tested (micrograms per plate)
First Experiment (no preincubation) and second experiment (preincubation)

- ◊ TA98 0, 20, 100, 500, 2500 and 5000
- ◊ TA100 0, 20, 100, 500, 2500 and 5000
- ◊ TA1535 0, 20, 100, 500, 2500 and 5000
- ◊ TA1537 0, 20, 100, 500, 2500 and 5000

- Statistical Methods: Not specified

Remarks Field for Test Conditions

- ◊ Positive controls
- ◊ Without activation
  - o AAC: TA1537
  - o NPD: TA98,
  - o MNNG: TA1535, TA100
- ◊ With activation
  - o 2-Aminoanthracene: all strains
- ◊ Solvent: aqua dest.
- ◊ Study generally follows OECD 471 differences include only four strains of bacterial used where five is recommended and AAC used as sole positive control under activation conditions.

Results

- Result: No dose-dependent increase in the number of revertants in any bacterial strain in the presence or absence of metabolic activation. Positive controls demonstrated the sensitivity of the test system
- Cytotoxic Concentration: No cytotoxicity observed at any level
- Genotoxic Effects: Not genotoxic under these conditions

Remarks Field for Results: No visible precipitation was observed at any concentration. No positive responses (doubling of the control mutation rate) were observed. The material was water soluble

Conclusions

Remarks field: No genotoxic activity. Study well conducted. Documentation is fair.

Data Quality

- Reliability: Klimisch Code 2 Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with GLP standards. Supporting studies increase the reliability of the conclusion.

References

Report on the Study of 1,3,5-Trioxane (ZST Test Substance No.: 88/164) in the Ames Test. Project 40M0164/884027. BASF AG. Department of Toxicology, Z 470, Ludwigshafen/Rhein, Germany (1988)

Other

This study is supported by a single-plate test from another laboratory using five strains of bacteria (TA98, TA100, TA1535, TA 1537 and TA1538) exposed with and without S-9 activation at eight concentrations of Trioxane from 0.5 to 5000 micrograms per plate. No mutagenic response was recorded in this study.[1]

Additional support comes from two additional published studies in prokaryotes which produced negative results In a triple plate test, with preincubation in the presence and absence of S-9 with strains TA97, TA98, TA100 and TA1535 at concentrations from 100 to 10000 micrograms per plate, Trioxane was reported to be without mutagenic activity.[2] The mutagenic activity of Trioxane was investigated in five strains of *S. typhimurium*: TA1535, TA1537, TA1538, TA98 and TA100 with and without activation by liver microsomes (induced with Aroclor 1254). It displayed no mutagenic activity under any of these conditions.[3]

References for Supporting Studies

1. Mutagenicity Evaluation of C-120 in the Ames Salmonella/Microsome Plate Test. Litton Bionetics Project # 20988. Sponsored by Celanese Corporation. 1980.
2. Zeiger, E, Anderson, B, Haworth, S, Lawlor, T, Mortelmans, K. Salmonella mutagenicity tests. 4 Results from the testing of 300 chemicals. Environ Mol Mutagen 11(Suppl 12):1-158,1988.
3. Kowalski, Z, Spiechowicz, E, And Baranski, B. Absence of mutagenicity of trioxane and dioxolane in Salmonella typhimurium. Mutat Res; 136 (3). 1984. 169-172

Mammalian Cell Transformation Assay, *in vitro*

Type	**Mammalian Cell Transformation Assay, *in vitro***
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3 C-235

Method

- Guideline: None specified
- GLP: No
- Year: 1981
- Cell Type:
  - ◊ C3H 10T-1/2 clone 8
  - ◊ Six replicates at each concentration in 60 mm dishes, 11-day incubation
  - ◊ Six replicates at each concentration in T-25 flasks, 11-day incubation
  - ◊ Six replicates at each concentration in 60 mm dishes, 38-day incubation
  - ◊ Six replicates at each concentration in T-25 flasks, 38-day incubation
- Concentrations tested:
  Concentrations tested (micrograms per ml)
  - ◊ 11-day incubation: 0, 1, 10, 100, 500, 1000, 5000, 10000 and 20000
  - ◊ 38-day incubation: 0, 1, 10, 100, 500, 1000, 5000, 10000 and 20000
  - ◊ 24- hour exposure to test material
  - ◊ 60 mm dished sealed in jar to reduce loss of test material
  - ◊ Vehicle: water or culture media
- Statistical Methods: Not specified

Remarks Field for Test Conditions
- ◊ Positive control: Benzpyrene
- ◊ Cultures re-fed on regular schedule
- ◊ Colonies examined macroscopically and microscopically.
- ◊ 300 cells per flask or dish
- ◊ Colony-forming potential determined at 11-day interval
- ◊ Foci transformation potential determined at 38-day interval

Results

- Result: No increase in the number of transformed colonies or transformed foci was noted at any concentration of test material. Positive controls demonstrated the sensitivity of the test system
- Cytotoxic Concentration: Severe cytotoxicity observed above 10000 micrograms/ml, slight dose-dependent cytotoxicity observed at 2000 microgram per ml and above.
- Genotoxic Effects: Not genotoxic under these conditions

Remarks Field for Results: The test material was water-soluble; precipitation was not recorded.

Conclusions

Remarks field: No genotoxic activity. Study was well conducted. Documentation is good.

Data Quality

- Reliability: Klimisch Code 2. Reliable with restrictions, study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with GLP standards.

References

Mammalian Cell Transformation Assay, University of Minnesota Experimental Pathology Laboratory, submitted to Celanese Corporation, dated 8/27/81.

Mouse lymphoma forward mutation assay

Type	**Mouse lymphoma forward mutation assay**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3

Method

- Guideline: None specified
- GLP: No
- System of Testing: Non-bacterial
- Year: 1980
- Species/Strain: Mouse lymphoma L5178Y TK+/-
- Metabolic activation:
  Tested with and without
  ◊ Rat liver S-9
  ◊ Aroclor 1245 induced
  ◊ Final concentration 5% S-9 in cell suspensions
- Concentrations tested: 0, 0.313, 2.5, 6.25, 12.5 and 15 mg/ml for both non-activation and activation conditions in first trial; 0, 0.156. 0.625, 2.5, 5.0 and 7.5 mg/ml in the second trial under activation conditions only.
- Statistical Methods: Simple ratio criteria

Remarks Field for Test Conditions
◊ Independent repeat of activation conditions only
◊ Triplicate plates for counting mutant colonies
◊ Solvent, water
◊ Negative control, water
◊ Positive controls
  - Without activation - EMS
  - With activation - DMN

Results

- Result: No dose-dependent increase in the number of mutants was observed in the absence of metabolic activation; however, the number of mutants was found to increase in the presence of metabolic activation. Positive controls demonstrated the sensitivity of the test system.

- Cytotoxic Concentration: No cytotoxicity observed under non-activation conditions. Under activation conditions, cytotoxicity was dose-dependent with moderate cytotoxicity observed at the lowest dose and extensive cytotoxicity reported for the highest concentrations.

- Genotoxic Effects:
  - ◊ No genotoxic activity under non-activation conditions
  - ◊ Concentration-dependent mutational activity under activation conditions.

Remarks Field for Results
- ◊ Material was soluble in water
- ◊ Under activation conditions, 4 fold increases in mutant frequency were observed at the moderately-toxic 0.156 mg/ml and the frequence increased to the 10 to 20 fold range at highly toxic concentration of 5 and 7.5 mg/ml.
- ◊ The positive control (DMN) under activation gave lower than expected mutational frequencies in the first trial but normal frequencies in the second trial indicating that the S9 activity was adequate.

Conclusions

Remarks field
- ◊ No genotoxic activity under non-activation conditions
- ◊ Concentration-dependent mutational activity under activation conditions.
- ◊ Mutational activity appeared to correlate with cytotoxicity suggesting metabolism by S-9 to active material.
- ◊ Study was well conducted, although there was no GLP certification the study appears to have been conducted using a GLP-quality protocol in a GLP compliant laboratory.

Data Quality

- Reliability: Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with GLP standards.

References

Mutagenic Evaluation of C-120 in the Mouse Lymphoma Forward Mutation Assay. Submitted to Celanese Corporation. Litton Bionetics, Inc, Kensington Maryland. LBI Project No. 20989, November 1980.

Genetic Toxicology *in vivo*

Mouse micronucleus assay

Type	Mouse micronucleus assay
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3 Source: Nitrogen Plant in Tarnow, Poland

Method

• Guideline	None specified
• GLP	No
• Year	1983
• Species/Strain	Mice/ BALB/c
• Sex	Male
• Route of Administration	IP Injection in water
• Doses	0, 2125 and 4250 mg/kg split into two doses given 24 hours apart
• Exposure Period	Approximately 30 hours
• Statistical Methods	The Wilcoxon test was used for statistical analysis of results. A positive response was defined as consisting of an increase in the incidence of polychromatic erythrocytes with micronuclei to at least twice the negative control value and a significance of at least $p \leq 0.05$.

Remarks Field for Test Conditions	◊	Age at Study Initiation	Seven to eight weeks
	◊	Number of animals per dose	Four per group. The dose was split into 2 and injected at 24-hour intervals. Animals were sacrificed 6 hours after the second dose of test material. Positive control groups contained three animals each.
	◊	Rationale for Animals	The OECD guideline specifies rats of each sex unless it has been shown in other studies that there is no sex difference in toxicity. The toxicity of Trioxane is known to be similar in males and females

◊ Control Groups and Treatment
- Negative Control: Water injection
- Positive Control: Mitomycin C in water. In a range of 0.65-5.2 mg/kg

◊ Clinical Observations
- None reported

◊ Criteria for Evaluating Results
- See statistical methods.

◊ Criteria For Selection of MTD
- Not specifically stated but the total high dose was half the "approximate lethal dose".

◊ Differences from Current OECD Guideline

This study varies from the current OECD 474 guideline. The significant variations are:
- The guideline specifies 5 animals/group
- The criteria of bone marrow toxicity at the high dose may not have been met; however, the "limit test" dosing of 2000 mg/kg was exceeded. The suggested number of dose levels is also irrelevant in light of the limit dose being met.

Results

- Genotoxic Effects: None indicated.

Remarks Field for Results

◊ Induction of Micronucleated Cells

	Trioxane		
Dose (mg/kg)	n	Number PCE examined	Incidence of micronuclei in PCE (% ± sem)
0	4	8000	0.42 ± 0.20
2125	4	8000	0.39 ± 0.20
4250	4	7550	0.47 ± 0.25
	Positive Control Mitomycin C		
0.65	3	5650	1.73 ±0.57
1.30	3	5660	3.00 ± 0.18
2.60	3	2800	3.47 ± 0.34
5.20	3	2300	2.24 ± 0.74

◊ Mortality — No mortality reported

Conclusions

◊ There was no induction of micronucleated polychromatic cells associated with administration of Trioxane.

Remarks field

◊ The high dose of dioxolane was stated to be toxic to the animals by the authors.

Data Quality

- Reliability: Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint.

Reference

Przybojewska B, Dziubautowska E, Kowalski Z. Genotoxic effects of dioxolane and trioxane in mice evaluated by the micronucleus test. Toxicol Lett 21:349-52 (1984)

In Vivo Unscheduled DNA Synthesis (UDS) Assay

Type	**In Vivo Unscheduled DNA Synthesis (UDS) Assay**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3 99.9% Batch 65-3997
Method	
• Guideline	OECD 486 (using revised draft guideline dated March 1996)
• GLP	Yes
• Year	1997
• Species	Rat
• Strain/Sex	Wistar (Chbb : THOM; SPF) male
• Route	Oral gavage
• Vehicle	Water
• Dose Levels	0, 250, 5000, 1000 and 2000 mg/kg
• Dosing Duration	Single dose
• Cell Harvest Time	4 and 18 hours after dosing
• Statistical Methods	Not conducted since negative result

Remarks Field for Test Conditions

◊ Concentration of test substance determined analytically in vehicle
◊ High dose is maximum recommended under OECD 486
◊ Rats weighed mean of 258 grams at time of dosing, age not specified
◊ Three animals per group (dose level and harvest time)
◊ Positive control 50 mg/kg 2-acetylaminofluorene (in corn oil)
◊ Hepatocytes harvested by collagenase perfusion 4 and 18 hours after dosing
◊ After attachment, cells incubated 4-hours with tritiated-thymidine
◊ Cells washed and incubated 14-hours longer with cold thymidine (chase)
◊ Cells coated with photo emulsion, exposed 19 hours and developed
◊ 100 random cells per animal counted for nuclear grains

Results

- Result: No treatment-related increase in the number of cells under repair
- Cytotoxic Concentration: > 2000 mg/kg
- Genotoxic Effects: Not genotoxic under these conditions

Remarks Field for Results

Cell viability

Dose	Viability (percent)	
	4-Hour Harvest	18-Hour Harvest
0	84	84
250	87	84
500	86	86
1000	82	84
2000	81	88
Positive Control	89	79

DNA Repair Activity

Dose	Percent cells in repair	
	4-Hour Harvest	18-Hour Harvest
0	8.67	7.33
250	Not examined	Not examined
500	10.0	12.0
1000	8.67	11.7
2000	10.0	6.33
Positive Control	78.7	84.7

Conclusions

Remarks field: No genotoxic activity. Study was well conducted. Documentation is very good. Study exceeds requirements of current OECD 486 in that determination of cells in repair was further segregated into 1). Percentage cells in repair with net nuclear grain count greater than or equal to zero. and 2). Percentage cells in repair with net nuclear grain count greater than or equal to five.

Data Quality

- Reliability: Klimisch Code 1. Reliable without restriction, study meets GLP standards and/or most requirements

References

In Vivo Unscheduled DNA Synthesis (UDS) Assay with 1,3,5-Trioxane in Rat Hepatocytes, Single Oral Administration. Department of Toxicology, BASG AG, Ludwigshafen. 12 March 1996.

Other

Male Rat Dominant Lethal, Oral

This study is the same as found in the Reproductive toxicology section. There it is listed as Dominant lethal, oral dosing and is found on page 87 of this document. The design of this study gives both genetic toxicity and reproductive toxicity information; thus, it is listed in both sections.

Male Rat Dominant Lethal, Inhalation

This study is the same as found in the Reproductive toxicology section. There it is listed as Dominant lethal, inhalation dosing and is found on page 90 of this document. The design of this study gives both genetic toxicity and reproductive toxicity information.

Reproductive Toxicology

Dominant Lethal, Oral Dosing

Type	**Dominant Lethal, Oral Dosing**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3
Method	
• Guideline	None, basically in accord with OECD 478
• GLP	No
• Year	1984
• Species	Rat
• Strain	Albino, Wistar
• Route of administration	Oral Gavage
• Doses	0, 850, 1700 mg/kg
• Sex	Male
• Number of Animals/group	10
• Vehicle	Water

Remarks Field for Test Conditions	◊ Age at Study Initiation	3 ½ to 4 months; weight 300 to 320 g
	◊ Doses	0, 850 and 1700 mg/kg/day
	◊ Dosing	Males only
	◊ Dosing Schedule	Five days a week
	◊ Dosing Duration	Eight weeks
	◊ Mating Interval	Weekly
	◊ Mating Ratio	2:1 females:males
	◊ Variations from OECD 478 Protocol Guideline	Guideline suggests that the number of males should be sufficient that 30-50 pregnant rats be evaluated at each time interval. In this study, 14 to 20 pregnant females were evaluated at each time; however, more time periods than typical were evaluated in this study. Current guideline suggests three dose levels; only two were used in this study.

Results

- Result: No evidence of dominant lethal effect

- Dominant Lethal Rate

	Dominant Lethals per Female (by week)							
Dose	1	2	3	4	5	6	7	8
0 mg/kg	0.84	0.83	1.45	1.29	0.89	1.11	0.93	0.67
850 mg/kg	1.11	1.06	0.39	0.26	0.35	1.17	0.44	0.37
1700 mg/kg	0.44	1.56	0.85	0.61	0.85	0.95	0.71	0.88

Number of deaths at each dose level

Dose	Mortality
0 mg/kg	0/10
850 mg/kg	0/10
1700 mg/kg	0/10

Remarks Field for Results

◊ Time of death: No deaths

◊ Clinical Signs: Behavior and appearance did not differ to any significant degree from controls

◊ Body Weights

Dose (mg/kg)	Body Weight gain (percent control gain)
850	50%
1700	42%

◊ Organ Weights: Absolute and relative weights of liver, kidney and spermatic vesicle were increased at both dose levels

◊ Necropsy and Microscopic Findings: Necropsy finding are not discussed. Histopathology was performed on the testes. Focal necrosis of the seminiferous epithelium was reported in 1/10 control, 3/10 850 mg/kg males and an unspecified number of high dose males. It was reported that the testicular lesions were bilateral in 3/10 high-dose males. Severity was dose dependent.

◊ Target Organs	Testes by histopathology but no change in fertility. Other organ weights were altered but there is no histopathology for confirmation. No changes in testes weight resulted from dosing.
◊ Criteria for Dominant Lethal Effect	Investigators used early resorptions as a measure of dominant lethal effect; however, neither implants per female, live fetuses per female nor preimplantation loss per female was affected by treatment.

Conclusions

Remarks field	Oral exposure of male rats to 859 or 1700 mg/kg Trioxane per day did not have any significant effects on the fertility rate as measured by the number of preimplantation loses, dead implants or live fetuses. Trioxane did not produce a dominant lethal effect by oral administration under these conditions The study appears to have been well conducted and is similar to current OECD guideline. Based on reduction in body weight gains, organ changes and histopathology the high-dose level produced signs of toxicity indicating the study is valid regarding achieving a systemically toxic dose.

Data Quality

• Reliability	Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with GLP standards. Study design and reporting meets current EPA/OECD guidelines with minor exceptions.

References

Baraânski, B; Stetkiewicz, J; Czajkowska, T; Sitarek, K; Szymczak W. Mutagenic and gonadotoxic properties of trioxane and dioxolane. Medycyna Pracy 5: 245-255 (1984)

Other

These results are supported by an inhalation study that is reported in the same publication

Dominant Lethal, Inhalation Dosing

Type	**Dominant Lethal, Inhalation Dosing**
Test Substance	1,3,5-Trioxane CAS Number: 110-88-3
Method	
• Guideline	None, basically in accord with OECD 478
• GLP	No
• Year	1984
• Species	Rat
• Strain	Albino, Wistar
• Route of administration	Inhalation
• Doses	0, 2500 mg/m^3
• Sex	Male
• Number of Animals/group	14
• Duration of Dosing	12 Months
• Vehicle	Air
• Statistical Methods	Kruskal-Wallis Test followed by non-parametric tests for groups with and without normal distribution.

Remarks Field for Test Conditions	◊ Age at Study Initiation	3 ½ to 4 months; weight 300 to 320 g
	◊ Doses	0 and 2,500 mg/m^3
	◊ Dosing	Males only
	◊ Dosing Schedule	Five hours a day for five days a week
	◊ Dosing Duration	12 Months
	◊ Mating Interval	At end of study for 1 week duration
	◊ Mating Ratio	2:1 females:males
	◊ Variations from OECD 478 Protocol Guideline	Guideline suggests that the number of males should be sufficient that 30-50 pregnant rats be evaluated at each time interval. In this study, 20 to 22 pregnant females were evaluated. Current guideline suggests three dose levels; only one was used in this study

◊ Other

Concentration of test material measured by chromatography using a published procedure.

2500 mg/m^3 is equal to 580 mg/kg/day assuming a 270 ml/min minute volume, a 350 gram rat and 100% absorption.

Results

- Dominant Lethal Rate

Dose	Effect (per female)					
Trioxane (mg/$m^{3)}$	Number Pregnant	Live Fetuses	Dead Implants	Number Implants	Corpora Lutea	Preimplant Loss
0	20	11.4	0.6	12	13.7	1.8
2500	22	12.2	0.64	12.8	14.0	1.1

- Number of deaths at each dose level

Dose	Mortality
0 mg/m3	0/14
2500 mg/m3	0/14

Remarks Field for Results

◊ Time of death — No deaths

◊ Clinical Signs — Not reported

◊ Body Weights — Not reported

◊ Organ Weights — Not reported

◊ Necropsy and Microscopic Findings — Testicular histopathologic effects were similar in control and treated group with regard to leydig cell pathology. Seminiferous tubule pathology was not reported for rats exposed to Trioxane by inhalation.

◊ Target Organs — None reported

Conclusions

Remarks field	Prolonged exposure of male rats to 2500 mg/m^3 Trioxane by inhalation did not have any significant effects on the fertility rate of these animals, with regard to the measured parameters which were the number of pregnant females, the number of females mating with males, the average litter size, average implantation numbers, pre-implantation losses, and corpus lutea. Trioxane did not produce a dominant lethal effect by inhalation at this concentration. Results are consistent with other data for the material. Since investigation of adverse testicular effects was a primary consideration of the study, and adverse effects on the testes were not reported, it is likely that 2500 mg/m^3 represents a NOAEL for testicular histopathology and for functional effects.

Data Quality

• Reliability	Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with GLP standards. Study design and reporting meets current EPA/OECD guidelines with minor exceptions.

References Baranski, B; Stetkiewicz, J. Evaluation of Mutagenic and Gonadotoxic Properties of Trioxane and Dioxolane. Medycyna Pracy:35 245-255 (1984)

Other These results are supported by an oral study reported in the same publication.

Repeated Dose Toxicity, 7-Week Oral with Estrous Cycle Determination

Type	**Repeated Dose Toxicity, 7-week Oral**
Test Substance	Trioxane CAS Number: 110-88-3

Method

• Guideline	None
• GLP	No
• Year	1990
• Species	Rat
• Strain	Wistar/Nofer Institute Breeding Colony
• Route of administration	Oral gavage
• Duration of Test	Seven weeks dosing, 13 weeks total
• Doses	0, 190, 580, or 1160 mg/kg/day
• Sex	Female
• Exposure Period	Once daily
• Frequency of Treatment	Five days a week Seven weeks
• Number of Animals/group	Ten or twelve
• Control Group and Treatment	Twelve animals dosed with vehicle
• Vehicle	Distilled water
• Post-Exposure Observation Period	5 weeks
• Statistical Methods	One-way or two-way analysis of variance and Scheffe's test for multiple comparisons.

Remarks Field for Test Conditions	◊ Age at study initiation	Four months
	◊ Number of animals per Sex per dose	▪ Ten per group at 190 and 580 mg/kg ▪ Twelve animals in the group at 1160 mg/kg
	◊ Satellite groups	None
	◊ Housing	Not reported
	◊ Clinical observations performed and frequency	▪ Body weights were measured weekly. ▪ General health and behavior, interval not reported ▪ Vaginal smears for the first 14 days of exposure, for the 14 days of the sixth and seventh week of exposure, and for the 14 days of the forth and fifth week after ending exposure (11th and 12th weeks of study).
	◊ Terminal observation	Body weights, general health
	◊ Histopathology	None reported

Results

- NOAEL
  - ◊ 580 mg/kg/day for estrous cycle effects
  - ◊ Not found for body weight gain
- LOAEL
  - ◊ 1160 mg/kg/day for effects on estrous cycle
  - ◊ 190 mg/kg/day for body weight gain
- Mortality

No deaths reported.

- Toxic Response

Dose	Effects
190 mg/kg	Significant reduction in body weight gain only at end of dosing period
580 mg/kg	Significant reduction in body weight gain only at week 6, 7 and 8 of study.

1160 mg/kg	Significant decrease in body weight gain reported at weeks 4 to 8, with recovery of body weight gain within two weeks after cessation of dosing. Statistically significant increase in the length of the estrous cycle after 6 to 7 weeks of exposure. Length of estrous cycle returned to normal after dosing was stopped. Behavioral changes were noted in this group it is stated in the publication: "The animals from the 1.16 gm/kg group at the end of the exposure, exhibited changes in appearance and behavior. They sat curled up in the cage corner with ruffles hair coats, they squealed when taken to hand by the experimenter and had sanquineous discharge from the nose. After cessation of exposure these disorders disappeared during the subsequent two weeks and their body weight leveled up to that of the control animals"

Remarks Field for Results

◊ Body Weights ◊ Provided as weekly bar chart in publication

◊ Estrous Cycle Length

	Cycle Length (days)		
Dose (mg/kg)	Week 1-2	Week 6-7	Week 11-12
0	4.6 ±1.4	4.3±0.4	4.8±1.2
190	4.3±0.5	4.4±0.9	5.0±1.6
580	5.1±1.6	5.4±2.8	4.5±0.6
1160	5.2±2.1	6.1±2.5*	5.1±2.8

◊ Necropsy Findings None Reported

Conclusions

Remarks field

◊ The authors conclude: "Based on the findings of this study, it seems unlikely that the occupational exposure of women to Trioxane at concentrations not inducing systemic intoxication, can produce alteration in their ovarian functions"

◊ The study design and conduct appear sound and the systemic toxicity produced is similar to that found in other studies on this material.

Data Quality

- Reliability

Klimisch Code 2 Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with GLP standards. The similarity of the results with supporting studies confirms reliability.

References

The effect of oral exposure to trioxane on the oestrous cycle in rats. Sitarek K; Baraânski B. Pol J Occup Med; VOL 3,1990, P209-13

Other

This study is supported by

- ◊ A 28-day gavage study conducted by Hoechst AG in male and female rats where a low degree of systemic toxicity was found at 1000 mg/kg after 28 days exposure and 200 mg/kg was found to be a NOEL.[1]
- ◊ A 4 or 7-month long oral gavage study in male rats. In this study groups of 8-10 rats were administered Trioxane as a water solution, 5 days a week. The high dose was 850 mg/kg and the duration of dosing was 4 months. Doses of 213 or 106 mg/kg were administered for 7 months. Body weight gain was similar for all dosed groups as compared to controls. The 850 mg/kg (four month duration for this group) did not affect body weigh gain after 4 months of administration. No specific organ effects were reported and it was concluded that the mortality rate did not show accumulated toxic effects of Trioxane. Examination of the limited data presented suggest that 213 mg/kg was (or is near) a NOAEL for Trioxane after 7-months of administration.[2]

References for supporting studies

1. Trioxane. Subakute orale Toxizitat (28 Application in 29 Tagen) an SPF-Wistar-Ratten. Hoechst AG Central Toxicology Report number 90.0513 22 May 1990
2. Experimental studies of the toxic effects of 1,3,5-trioxane and 1,3-dioxolane. II. Cumulation of toxic effect. Czajkowska T; Krysiak B Med Pr; VOL 38, 1987, P244-9

Developmental Toxicology

Developmental Toxicology, Oral

Type	**Developmental Toxicology, Oral**
Test Substance	Trioxane 99.77 % Trioxane as a 20% aqueous solution Stability data provided and dosage solutions analyzed

Method

• Guideline	OECD 414 "Teratology" dated 12 May 1981 EC 88/302/EWG US EPA OPPTS Series 83-3 "Teratology Study" November 1984 Japanese MAFF 4200, 28 January 1985
• GLP	Yes
• Year	1998
• Species	Rat
• Strain	Wistar, Hoe: WISKf(SPF71)
• Route of administration	Oral gavage, as 20% solution in water
• Doses	0, 100, 315, 1000 mg/kg
• Sex	Female, pregnant
• Exposure Period	Days 7 to 20 of pregnancy
• Frequency of treatment	Daily
• Control Group	Water only
• Duration of test	14 days
• Statistical Methods	Two-sided comparison of high dose group followed by one-sided comparison of low-dose group. Fetal data analyzed using multivariate statistics to select the relevant dose groups followed by sequential comparisons with the high-dose group. Feed data analyzed using Wilcoxon rank sum test. Body weights of dams used univariate evaluation by t-tests. Fetal mean litter values were compared using the test statistic of Wilks for multivariate comparisons, and the t-test for univariate comparisons. The number of corporea leuta, implantation sites and live fetuses, and quotas of dead embryonic primordia undergoing resorption in the animals were analyzed using one-sided Wilcoxon tests. Fisher's Exact Test was used to analyze findings at autopsy, body cross-section and skeletal examination; separate analyses were conducted for the individual fetal data and for the litter data at significance levels of 5% and 1%.

Remarks Field for Test Conditions	◊ Age at Study Initiation	8-10 weeks at start of in-house breeding procedure
	◊ Number of animals per group	23 mated females per dose group
	◊ Vehicle	Deionized water
	◊ Clinical Observation Performed and Frequency	Behavior and health observed twice daily. Body weights determined on days 1,4, 7, 14, 17, 19 and 21 of gestation. Food consumption determined between days 1-4, 4-7, 7-10, 10-14, 14-17, 17-19, and 19-21 of gestation.
	◊ Mating Procedures	Virgin females in pre-estrus or estrus phase were mated 1:1 with males overnight. After detection of sperm in vaginal smears, females were housed individually and presumed pregnant. The day of sperm detection was defined as day-1 of gestation.
	◊ Maternal Parameters Assessed During Study	Body weight, feed consumption and clinical signs, corpora lutea, implantations, uterus weight
	◊ Fetal Parameters Assessed During Study	Litter size, placental weight, gross malformations, fetal crown-rump length, fetal body weight, sex ratio, body cross sections, skeletal examination
	◊ Organs Examined at Necropsy	Full list not given, findings included – ovary, cervix, kidney and uterus
	◊ Dose Selection	A dose-range-finding study was conducted using groups of 4 mated Wistar rats dosed at 500 or 1000 mg/kg per day from day 7 - 20 of pregnancy and were killed on day 21. The uterus was opened and the number of live and dead fetuses and the number of conceptuses undergoing resorption were determined. The fetuses were examined for gross major anomalies. Fetal body weight and crown-rump length were recorded. Two animals from the 1000 mg/kg group showed slight loss of body weight from day 7 - 10 and exhibited increased numbers of retarded fetuses. No compound-related effects were observed in the other animals. Based on these results the dose levels of 0, 100, 315 and 1000 mg/kg body weight per day were selected for the present study. Testing of dose levels greater than 1000 mg/kg body weight is not necessary according to current OECD Guidelines.

Results

- NOAEL & LOEL for Maternal Toxicity
  NOAEL = not established
  LOEL = 100 mg/kg/day body weight gain
- NOAEL & LOEL for Developmental Toxicity
  NOAEL = 100 mg/kg/day
  LOEL = 315 mg/kg/day retarded ossification
- Actual Doses Received
  0, 100, 315, 1000 mg/kg/day
- Maternal data
  Body weight gain and food consumption was significantly decreased in high-dose animals from day-10 to the end of the study. Mean corrected body weight gain (day-21 body weight minus gravid uterus weight) was decreased with statistically significance $p \leq 0.05$ for all dosed groups. Control 33.6 g., low dose 27.13 g., intermediate dose 28.96 g, and high dose 18.88 g. Although the dose dependency is not clear, there appears to have been reduction in maternal weight gains at all dose levels indicating some maternal toxicity at even the low dose. No compound-related effects were observed at necropsy. Gravid uterus weights were comparable in all groups. One animal in the high dose and one animal in the low dose group showed only implantation sites at cesarean section. Based on historical data and lack of dose-dependency, this was not considered compound related. Five dead fetuses were observed in the high-dose group and this was statistically significant compared to the control group. A compound-related effect cannot be ruled out for fetal death at the high dose.
- Fetal data
  - ◊ Cesarean Data
    Litter size was comparable in all groups. In the high-dose group, fetal body weights and crown-rump length were decreased, and placental weights were increased. Sex ratio was unaffected by treatment.
  - ◊ External and visceral effects
    The incidence of retarded fetuses was 6/123, 6/117, 8/139 and 29/131 for control to high dose respectively. This was statistically significant in the high-dose group compared to control. Two high-dose fetuses showed tail aplasia that may be compound related. Other findings were within the historical control range.
  - ◊ Major skeletal defects
    Two high-dose fetuses showed tail aplasia, these same two also showed aplasia of the sacral vertebral arch, sacral vertebrae centers and first and second caudal vertebrae centers.

    One fetus from the low-dose showed fusion of the exoccipital bone with the first cervical vertebrae and dysplasia of the exoccipital bone. One fetus from the high-dose group showed dysplasia of the exoccipital bone. The incidence of these defects was within the historical control range of the rat strain.

- Fetal data

◊ Minor skeletal defects

	Dose Group			
Effect	0	100	315	1000
Longitudinally displaced, fused or fragmented sternebrae	12/123	3/117	1/139	17/131*
Wavy and or thickened ribs	12/123	14/117	56/139*	84/131*
Bent or shortened scapula	0/123	0/117	2/139	12/131*
Bent, shortened or dysplastic humerus	0/123	0/117	1/139	12/131*
Bent or shortened radius	0/123	0/117	0/139	5/131*
Fragmented thoracic vertebral centers	0/123	0/117	2/139	4/131

* = statistically significant

◊ Retard-ations

	Dose Group			
Effect	0	100	315	1000
Slight or non-ossification of skull bone	42/123	50/117	74/139*	80/131*
Weakly or non-ossified cervical vertebral arch	0/123	0/117	3/139	13/131*
Weakly ossified lumbar vertebral arch	0/123	0/117	2/139	13/131*
Weakly or non-ossified sacral vertebral arch	3/123	3/117	2/139	14/131*
Ossification of less than two caudal vertebral centers	26/123	50/117*	85/139*	98/131*
Weakly ossified ribs	0/123	1/117	3/139	9/131*
Weakly ossified metacarpal 2	0/123	0/117	0/139	5/131*
Non-ossified metacarpal 5	58/123	61/117	92/139*	86/131*
Non-ossified metatarsal 5	3/123	2/117	1/139	11/131*
Weakly or non-ossified sternebrae	18/123	42/117*	45/139*	70/131*
Non-ossified phalanx 3 of the 1st to 5th hindpaw toe	1/123	7/117*	3/139	18/131*

* = Statistically significant

◊ External and visceral effects

Retarded fetuses observed at body cross section

0	100	315	1000
0/116	2/109	1/125	17/121*

* = Statistically significant

- Statistical Results: Fetal data statistical determination by Fisher's Exact test (* indicates $p < 0.05$)

Remarks Field for Results: Low-dose retardations were not considered compound related by the study director due to lack of dose-response or historical range.

Parameter	Dose Group			
	0	100	315	1000
Pregnancies	20/23	21/23	21/23	21/23
Females with abortion	0	1	0	1
Corpora lueta (total)	280	287	316	298
Implantations (total)	246	245	284	276
Pre-implantation loss, mean percent	12.78	14.10	10.08	7.35
Post-implantation loss, mean percent	2.62	7.65	7.22	8.36
Live fetuses (total)	239	226	264	252
Total intrauterine deaths	7	19	20	24
Males %	48.1	59.7	55.7	50.8
Body weight (mean grams)	3.3	3.3	3.2	2.8*
Crown-rump length (mean mm)	35.1	35.0	34.5	32.8*
Placental weight (mean g.)	0.46	0.47	0.49	0.52
Uterus weight (mean g.)	60.40	57.57	62.57	59.47

Conclusions

Remarks field

Based on the results of this study, Trioxane is not considered a specific developmental toxin. The developmental NOEL was found to be 100 mg/kg/day while a maternal NOEL was not defined since corrected dam body weight gain was affected in all treated groups.

Neither deaths nor clinical signs were observed in any of the animals.

Gravid uterus weights, litter size and fetal sex rations were not affected. Fetal body weights and crown-rump lengths were decreased in the high-dose group whereas placental weights were increased. Early resorption was not affected by Trioxane administration. Five dead fetuses were observed in five litters at the high dose.

In the high-dose group, two cases of major defects were observed. The incidence of fetuses with minor defects was increased. Retarded ossification was observed in numerous bones.

In the mid-dose group, the incidence of minor defects was increased and retarded ossification of individual skull bones and caudal vertebral centers were observed.

In the low-dose group, no compound-related effects were observed by morphological observation.

Data Quality

- Reliability: Klimisch Code 1. . Reliable without restriction, study meets GLP standards and/or most requirements

References

Hofmann Th. Trioxan, Rat Oral Developmental Toxicity (Teratogenicity) study. Hoechst Marion Roussel, HMR Deutschland GmbH, Global Preclinical Development, Drug Safety, Study Number 97.0791 (1998).

Other

Supporting data comes from previous published developmental toxicity studies. In a developmental toxicity study of Trioxane, pregnant Wistar rats were given 770, 1550, or 3870 mg/kg Trioxane orally every other day from day 8 to 20 of gestation. Other rats included in this protocol were given 190 mg/kg Trioxane or 20 mg/kg Formaldehyde daily during gestational days 8 to 20. Dams were killed on gestational day 21 and necropsied. Placentas were examined for histopathological changes. The numbers of resorptions and live and dead fetuses were recorded. The fetuses were weighed and examined for malformations. Trioxane did not cause any deaths, but caused significant maternal toxicity as evidenced by decreases in body weight gain, feed intake, absolute liver and placenta weights and increases in relative kidney and adrenal weights. The 3890 mg/kg dose was associated with hydropic liver in dams. Trioxane induced dose dependent increases in the number of resorptions and decreases in the number of live fetuses and fetal body weight and length. The 770 to 3870 mg/kg every-other-day doses induced malformations in the brain, kidneys, and skeleton. The 770-mg/kg dose was associated with developmental effects without reported maternal toxicity. The daily 190-mg/kg dose did not cause any developmental effects. Trioxane administration was associated with fibrin deposits, inflammatory infiltration, and focal necrosis in the placentas. The authors conclude that Trioxane at sufficiently high concentrations causes fetal lethality, retards fetal development, and induces congenital malformations[1].

A study designed to examine the postnatal effects of Trioxane using the every-other-day dosing regime was conducted at 190, 580 or 1160 mg/kg/day from day 2 to 20 of gestation. In this study, the high dose was associated with high post-natal mortality and reduced maternal instincts. The mid-dose was reported to be associated with reduced active-avoidance acquisition of offspring at 5 months. The low-dose, 190 mg/kg/day, was reported to be a NOEL for behavioral developmental effects in the pups[2].

References for supporting studies

1. Sitarek, K, Baranski, B, Stetkiewicz, J, Stetkiewicz, I, Teratogenicity, Fetal and Placental Toxicity of 1,3,5-Trioxane Administered to Pregnant Female Rats. Polish Journal of Occupational Medicine 1:51-61, (1988).
2. Sitarek, K, Baraânski, B. Effects of maternal exposure to trioxane on postnatal development in rats. Pol J Occup Med 3:285-92 (1990)