Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

1,3-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether

EC Number: **202-987-5**

CAS Number: **101-90-6**

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Note on confidential information

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CONTENTS

1 PHYSICAL HAZARDS

This hazard class has not been evaluated.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1 Study 1: Seiler (1984a)

Male and female ICR-mice were treated orally with ^{14}C labelled resorcinol diglycidyl ether in a single dose of 500 and 1000 mg/kg bw (vehicle: 10% DMSO solution) (Seiler, 1984). Dose levels were equivalent to the ones used in the micronucleus test (see section 3.8.2.3). The animals were kept in metabolic cages, where they had access to water only during the experiment. Urine and faeces were collected for 1-4 h and analysed for metabolic products (the number of replicates was not reported). Urinary metabolites were then extracted either by ethylacetate at pH 7 and pH 2, or by XAD-2 adsorption. In addition, epoxidase hydrolase containing liver homogenates (S9) were incubated with resorcinol diglycidyl ether and measured remaining alkylating activity. Analysis of urinary and S-9 metabolites was by thin-layer chromatography on silica gel plates in acetone/hexane (1:1) as the most suitable solvent system.

Results were presented as overall data (male and female animals combined). Table 2.1-1 presents information on the urinary excretion (as a measure for oral uptake). Table 2.1-2 presents information on the quantitation of metabolites of resorcinol diglycidyl ether.

Table 2.1-1. Urinary excretion of resorcinol diglycidyl ether in ICR-mice (Seiler, 1984a).

Total dose (cpm x 10^{-6} /time point)	**Chemical dose (mg/kg bw)**	**Cpm ($x10^{-3}$) recovered from urine[a]**		
		60 min	**120 min**	**240 min**
0.04	1000	4.4 (10.5)	9.2 (22.1)	5.9 (14.2)
13.8	500		1950 (14.1)	-

[a] Figures in parentheses are % of applied dose

Table 2.1-2. Quantitation of urinary metabolites from resorcinol diglycidyl ether (Seiler, 1984a).

	Urinary metabolites (%)
Bis-epoxide	-
Diol-epoxide	-
Phenol-diol	4 (0.42)
Bis-diol	64 (0.19)
Unidentified polar (conjugates?)	21 (0.06)

Figures are given in % of amount applied in thin-layer plate, with the respective *r*1-values in brackets

The total amount of radioactivity recovered from urine collected up to 4 hours after a single oral dose of 1000 mg/kg bw was nearly 50% of the applied dose. Four per cent of the metabolites detected in the urine was the phenol-diol metabolite, 64% was the bis-diol metabolite and 21% of the metabolites could not be identified. No bis-epoxide or diol-epoxide was excreted.

In addition, three type of studies were then performed, the first one giving an indication of the time course of the disappearance of the alkylating activity, the second one showing the appearance and disappearance of certain intermediates in the course of metabolic processes and the third one allowing the calculation of some quantitative parameters of enzyme action. For these experiments, post-mitochondrial supernatant of rat liver homogenate (S9) was used at dilutions of 5-20% in PBS. No cofactors were added, since the only enzyme of immediate interest was epoxide hydrolase, which does not need NADPH for its action. Resorcinol diglycidyl

ether showed apparent first-order kinetics and a half-life of about 6 minutes. The diol-epoxide was formed as an intermediate before transformationinto the bis-diol.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 Study 1/2/3: Hine et al. (1958) - rat/mouse/rabbit

Rats (Long-Evans; male; number of animals per group not specified), mice (Webster; male; number of animals per group not specified) and rabbits (albino male; number of animals per group not specified) were exposed by intragastric administration to resorcinol diglycidyl ether (Hine et al., 1958). The compound was diluted with propylene glycol where necessary for ease of administration. Because of the large volume of the highest intragastric dose for rats, the suspension was given in two aliquots, three hours apart, to fasted animals. Information on the individual dose levels was not provided. Animals were observed during a 10 day postexposure observation period. Those that died were immediately subjected to necropsy, the survivors were killed for necropsy, and sections of their tissues were preserved in 10% formalin for histological examination. LD_{50} values were calculated by Hine et al. (1958) according to the method of Litchfield and Wilcoxon (1948) or the method of Weil (1952).

LD_{50}-values of 2570, 980 and 1240 mg/kg bw were reported for rat, mouse and rabbit, respectively. The lethality data per exposure group was however not presented for any of the tested species. There was moderate depression, slight dyspnea, and in surviving animals loss of weight and diarrhea observed.

3.1.2 Human data

N/A

3.1.3 Other data

N/A

3.2 Acute toxicity - dermal route

3.2.1 Animal data

3.2.1.1 Study 1: Westrick and Gross (1960), as cited in Gardiner et al., 1992 - rabbit

Rabbits (strain, sex and number of animals/group not specified) were dermally exposed for 7 hour to resorcinol diglycidyl ether (Westrick and Gross (1960), as cited in Gardiner et al., 1992). A non-occlusion condition was applied. The test substance was applied as a 60% solution in xylene. Limited details on the conduct of the study are available.

An LD_{50} of 2420 mg/kg bw (2.0 ml/kg bw) was reported. The lethality data per exposure group were however not presented. Also no details on clinical signs or other effects were presented.

3.2.1.2 Study 2: Westrick and Gross (1960), as cited in Gardiner et al., 1992 - rabbit

Rabbits (strain, sex and number of animals/group not specified) were dermally exposed to resorcinol diglycidyl ether (Westrick and Gross (1960), as cited in Gardiner et al., 1992). The individual dose levels were not specified. Limited details on the conduct of the study are available.

An LD_{50} of 744 mg/kg bw (0.64 ml/kg bw) was reported. The lethality data per exposure group were however not presented. Also no details on clinical signs or other effects were presented.

3.2.2 Human data

N/A

3.2.3 Other data

N/A

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

3.3.1.1 Study 1/2: Hine et al. (1958) - rat/mouse

Rats (Long-Evans; male; number of animals per group not specified) and mice (Webster; male; number of animals per group not specified) were exposed for 8 hours by whole body inhalation to resorcinol diglycidyl ether (Hine et al., 1958). Air was saturated with resorcinol diglycidyl ether at 30°C by passage through the liquids in two fritted glass bubbles connected in series. The attained saturated concentration was not analytically checked.

Information on the individual concentration levels was not provided. Animals were observed during a 10 day postexposure observation period.

No deaths or clinical effects were observed.

3.3.1.2 Study 3: Westrick and Gross (1960), as cited in Gardiner et al., 1992 - rat

Rats (strain, sex and number of animals/group not specified) were exposed for 4 hour to an aerosol of resorcinol diglycidyl ether (Westrick and Gross (1960), as cited in Gardiner et al., 1992). A concentration of 44.8 mg resorcinol diglycidyl ether (60% in xylene) per liter of air was applied. Limited details on the conduct of the study are available.

All animals died within 5 days postexposure.

3.3.2 Human data

N/A

3.3.3 Other data

N/A

3.4 Skin corrosion/irritation

This hazard class has not been evaluated. However, in support of the evaluation of the endpoint carcinogencity, the individual studies can be found below.

3.4.1 Animal data

3.4.1.1 Study 1: Hine et al. (1958)

Albino rabbits (sex and number not specified) were treated topically with resorcinol diglycidyl ether (Hine et al., 1958). Animals were clipped over the back and flanks, and four areas of the back designated for the test, two intact and two ssarified with wire mesh. The animals were immobilized in a multiple rabbit holder, and patches of gauze were secured over the areas with adhesive tape. The compounds were introduced under the patches, and the entire trunk of the rabbits enveloped in rubber dam. After 24 hours the rabbits were released, the chemical removed, and a first reading was made. After 72 hours, a second reading was done.
Resorcinol diglycidyl ether was moderately irritating to te skin with a score of 5 (out of maximal 8).

3.4.1.2 Study 2: Hine et al. (1958)

Albino rabbits (sex not specified; four animals) were treated topically with resorcinol diglycidyl ether (Hine et al., 1958). Four to five spots on each animal's back were depilated, and a different compound applied to each spot (in addition to resorcinol diglycidyl ether, also other chemicals were tested in this study). The chemicals were diluted with acetone if it was not practical to apply them undiluted. The materials remained on the skin for seven hour periods, after which they were removed with acetone. Readings were made every 24h, just prior to the next application. The degree of irritation was scored by the same method as for single application, in which the maximum score for erythema and edema is 8. Applications were made daily, except weekends, for a total of 20, or until death or eschar formation made further application impractical. The rabbits were weighed weekly, and after the final application representative aniamls were killed to determine the systemic effects. Suitable tissues were taken for histologic study.
Repeated application of resorcinol diglycidyl ether produced severe irritation. Three out of four rabbits died after seven applications. These animals showed dyspnea, rales, and nasal discharge, and signs of bronchopneumonia were found in all at necropsy. The study authors considered that death was due primarily to disability produced by the extreme irritation, rather than to any systemic effects of the chemical.

3.4.1.3 Study 3: Westrick and Gross (1960)

In a primary skin irritation test, 0.01 ml of 10% solution of resorcinol diglycidyl ether in acetone was applied topically to the skin of five rabbits (strain and sex not specified). No details on the exposure period and time points of evaluation were provided. Scar tissue formation was noticed in one animal. In the other four animals, a definite erythema and edema were observed.

3.4.1.4 Study 4: Westrick and Gross (1960)

Resorcinol diglycidyl ether (0.5 ml as a 60% solution in xylene) was applied to rabbit skin (strain, sex and number of animals was not specified) for 24 h. No details on time points of evaluation were provided. Severe irritation was noticed which progressed to necrosis.

3.4.2 Human data

N/A

3.5 Serious eye damage/eye irritation

This hazard class has not been evaluated.

3.6 Respiratory sensitisation

This hazard class has not been evaluated.

3.7 Skin sensitisation

This hazard class has not been evaluated.

3.8 Germ cell mutagenicity

This hazard class has not been evaluated. However, in support of the evaluation of the endpoint carcinogencity, the individual studies can be found below.

3.8.1 In vitro data

3.8.1.1 Study 1: Canter et al. (1986), NTP (1986)

A salmonella mutagenicity assay was performed using *Salmonella typhimurium strains*: TA98, TA100, TA1535, TA1537. Resorcinol diglyidyl ether was tested in 5 doses in DMSO using triplicate plates. Tests were repeated at least once; a chemical was not designated positive or negative unless the results were reproducible.

Metabolic activation was applied using Liver S9-mix from Aroclor 1254-induced male Sprague-Dawley rats and Syrian Hamsters. Positive controls were for -S9 mix: sodium azide (TA100, TA1535), 9-aminoacridine (TA1537), 4-nitro-o-phenylenediamine (TA98); +S9 mix 2-aminoanthracene (all strains). The applied dose resorcinol diglycidyl ether (87.9% purity) was 0-333 µg/plate (-S9) and 0-2000 µg/plate (+S9) for the initial study; and 0-100 µg/plate (-S9) and 0-1000/1500 µg/plate (+S9).

Outcome: With respect to cytotoxicity, there was a slight clearing of background lawn in the highest and sometimes second to highest dose tested.
With respect to the revertant mutations, the results were as follows:

- TA98: negative
- TA1537: negative
- TA1535: positive with and without metabolic activation
- TA100: positive without metabolic activation and with rat S9 mix; equivocal with hamster S9 mix

3.8.1.2 Study 2: Seiler (1984b)

Salmonella mutagenicity assays were performed according to Ames et al. (1975). Bacteria strain TA100 was used and 5.8 x 10^7 cells were seeded per plate. Bacteria were incubated for 6h under continuous shaking until the desired cell density was reached. The number of living cells plated was checked in the following manner: 0.1 ml of the appropriate dilution of the bacteria in isotonic saline was added to 2 ml of top agar containing an amount of histidine sufficient for the unlimited growth of all bacteria. Other growth conditions, e.g. biotin content, composition of base agar, and incubation temperature, were equal to those of the mutagenicity test, and colony counts were also performed after 72h incubation.

Metabolic activation was not applied. The mutagenicity effect of resorcinol diglycidyl ether (>98% purity) was tested at amounts of 0, 50, 100, 200, 500 and 1000 µg per plate. A positive control was not used, and details on the negative control was not specified.

Outcome: Positive. Cytotoxicity was observed at dose levels of 500 and 1000 µg/plate. The median number of revertant colonies per plate were 116, 438, 609, 772, 117, toxic, for the 0, 50, 100, 200, 500 and 1000 µg per plate exposure conditions, respectively.

3.8.1.3 Study 3: McGregor et al. (1988)

McGregor et al. (1988) investigated the gene mutation effect of resorcinol diglycidyl ether in mouse lymphoma cells (*tk* locus).

Exposure: Each exposed culture consisted of $6x10^6$ cells in a final volume of 10 ml culture medium in a 30-ml screw-cap plastic tube. This tube was incubated for 4 h on a horizontal axis roller drum rotating at 10 rpm. At the end of the incubation time, the cells were sedimented by centrifugation at 500 g.av. for 10 min, washed, and finally resuspended in 20 ml culture medium. These cell suspensions ($3x10^5$ cells/ml) were incubated for a 2-day expression period, the cell population density being adjusted back to 20 ml of $3x10^5$ cells/ml after 24 h. After 48 h, the cell population densities were estimated and culture volumes containing $3x10^6$ cells adjusted to 15 ml with culture medium, giving a cell population density of $2x10^5$ cells/ml.

Cloning efficacy: A 0.1 ml sample of the cell suspension was withdrawn and diluted 1:100. Three 0.1 ml samples (200 cells) of the diluted cultures were transferred to 30-ml tubes, mixed with 25-ml cloning medium containing 0.35% Noble agar and poured into 90 mm petri plates.

Mutant selection: Three aliquots (each containing 10^6 cells) of the remaining culture were distributed to 30-ml tubes, mixed with 20-ml cloning medium to give final concentrations of 0.35% Noble agar and 3 µg trifluorothymidine/ml, then poured into 90-mm petri plates.

Incubation: the agar was gelled at 4°C for 5-10 min, then the plates were incubated for 11-14 days in 5% CO2: 95% air at 37°C.

Colony counting: colonies were counted using an Artek 880 Automate Colony Counter, with the colony size discriminator control in the "off" position.

Calculations: Toxicity was expressed as either a reduction of cell population growth in suspension during the expression period or a reduction in cloning efficiency. A measure of the overall toxicity was the relative total growth (RTG), which is defined as: RTG = (total suspension growth x cloning efficiency) in dosed culture/(total suspension growth x cloning efficiency) in control culture. Mutant fraction (MF) was calculated as the number of mutant colonies formed/million viable clones.

Metabolic activation was not applied. EMS was used as positive control. L5178Y mouse cells were treated with resorcinol diglycidyl ether (unknown purity) in concentrations of 0, 0.25, 0.5, 1, 2, 4 µg/ml (1^{st} test) and 0, 0.125, 0.25, 0.5, 1 and 2 µg/ml (2^{nd} test).

Outcome: Positive. The mutant frequency (i.e. no. of mutant clones/million viable clones) were as follows: 60, 339, 783 761, lethal, lethal (1st test) for 0, 0.25, 0.5, 1, 2, 4 µg/ml resorcinol diglycidyl ether respectively and 35, 182, 369, 689, 982, lethal (2nd test) for 0, 0.125, 0.25, 0.5, 1 and 2 µg/ml resorcinol diglycidyl ether.

3.8.1.4 Study 4: McGregor et al. (1996)

McGregor et al. (1996) investigated the gene mutation effect of resorcinol diglycidyl ether in mouse lymphoma cells (*tk* locus). Briefly, the $tk^{+/-}$ heterozygote of L5178Y mouse lymphoma cells were cleansed of *tk* and *hprt* mutants before being used in experiments. Six million cells were exposed to the chemical for 4 hr. Two days after exposure, an aliquot was removed for treatment with 3 ng/ml TFT in soft agar for selection at the *tk* locus. After 7 days a sample was removed for treatment with 4 ng 6TG/ml in soft agar for selection at the *hprt* locus. The numbers of mutant colonies were counted at the end of each expression period. In parallel, an independent measurement of the cloning efficiency was made in soft agar in the absence of any selective agent. Mutant fractions were calculated from these parameters. Relative total growths were also calculated.

Toxicity was expressed as a reduction of either cell population growth in suspension during the expression period or cloning efficiency. A measure of the overall toxicity was the relative total growth (RTG), which is defined as follows: RTG = (total suspension growth x cloning efficiency) in dosed culture/(total suspension growth x cloning efficiency) in control culture. In each case, culture dilutions were made 1 day prior to the addition of the selective agents. Each time this was done, cells were counted and this number used in the

RTG calculation. Mutant fraction (MF) was calculated as the number of mutant colonies formed/million viable clones.

Metabolic activation was not applied. EMS was used as positive control. L5178Y mouse cells were treated with resorcinol diglycidyl ether (unknown purity) in concentrations of 0, 0.1, 0.4, 0.7 μg/ml (first experiment) and 0, 0.1, 0.2, 0.4 μg/ml (second experiment). The mutagenic responses at the *tk* and *hprt* loci were examined. The results are shown in Table 3.8.1.4-1.

Outcome: Resorcinol diglycidylether induced mutagenic responses at the *tk* locus at doses with RTG values greater than 10%. The positive control substance (250 ng/ml EMS), induced a clear mutagenic response at the tk locus in all the experiments with an average mutant fraction of 198 mutants per million viable cells. No reproducible or dose-related positive response at the *hprt* locus at 7 days of expression was observed upon exposure to resorcinol diglycidyl ether. At the hprt locus, the average mutant fraction for the positive control, EMS, was 131 mutants per million viable cells.

Table 3.8.1.4-1. Mutagenicity at the *tk* and *hprt* locus induced by resorcinol diglycidyl ether (McGregor et al., 1996).

Dose resorcinol diglycidyl ether (μg/ml)	***Tk*-locus**		***Hprt*-locus**	
	Mutant fraction (*tk*)	**Relative total growth (RTG)**	**Mutant fraction (*hprt 7 days*)**	**Relative total growth (RTG)**
1st experiment				
0	14	100	4	100
0.1	45	65	-	-
0.4	157	21	8	27
0.7	238	6	22*	7
EMS	197	74	125	69
2nd experiment				
0	21	100	12	100
0.1	48	88	7	72
0.2	99	58	4	53
0.4	173	38	16	38
EMS	157	84	79	63

* Data point not considered in analysis because of excessive toxicity

3.8.1.5 Study 5: Gulati et al. (1989)

Gulati et al. (1989) tested the chromosome aberration potential of resorcinol diglycidyl ether in Chinese hamster ovary cells.

Briefly, approximately 24 h prior to cell treatment, 1.2 x 10^6 cells were seeded per 75 cm^2 flask. A culture was established for each dose both with and without metablioc activation. A liver fraction (S9) prepared from Aroclor 1254-induced male Sprague-Dawley rats was used to provide exogenous metabolic activation. The final concentrations of the S9 fraction, NADP, and isocitric acid were 0.02 ml, 2.4 mg, and 4.5 mg, respectively per ml culture medium. For assays without metabolic activation, the testing approach was similar to the corresponding SCE studies (see section 3.8.1.7), except that cells were treated for about 10 h and BrdUrd was omitted. Colcemid was added 2-3 h prio to cell harvest by mitotic shake-off. The test protocol for assays with metabolic activation was also similar to the corresponding SCE studies except that BrdUrd was omitted and cells were harvested approximately 11 h after removal of S9 fractions. Colcemid was added 2 h prior to harvest. Slides were stained in 6% Giemsa for 5-10 min. One hundred cells were scored for each dose in early studies and 200 cells per dose in later studies. All slides except high-dose positive controls were coded. Only metaphase cells in which the chromosome number was between 19 and 23 were scored. The chromosome number was recorded for each cell and chromosome or chromatid type aberration were classified into three categories: simple (breaks, fragments, double minutes), complex (interchanges, rearrangements), and other (pulverized, more than ten aberrations/cell). Positive results in

initial tests were confirmed by additional tests. If both –S9 and +S9 studies gave a positive response and required confirmation, they were done sequentially (-S9 first). If the –S9 repeat was positive, the repeat +S9 study was not always performed.

Resorcinol diglycidyl ether (>87.9% purity) was tested in concentrations of 0, 0.5, 1.6, 5, 16 µg/ml (-S9) and 0, 5, 16, (25 only in 2nd test), 50 µg/ml (+S9). As solvent, DMSO was used. Positive controls were mitomycin C (-S9) and cyclophosphamide (+S9).

Outcome: Treatment of cultured CHO cells with resorcinol diglycidyl ether produced highly significant increases in chromosome aberrations, with and without metabolic activation. The % cells with aberrations were as follows (* indicates statistical significance):

- 3, 1, 4, 14*, 61* (-S9, 1st test)
- 0, 5*, 6*, 40*, 69* (-S9, 2nd test)
- 3, 3,10, 58* (+S9, 1st test)
- 3, 5, 8, 6, 27* (+S9, 2nd test)

3.8.1.6 Study 6: Seiler (1984b)

A chromosome aberration test was performed with chines hamster ovary cells. For the test, $2x10^5$ cells were seeded into 25 cm^2 culture flasks and incubated for 24 h. After this period, resorcinol diglycidyl ether was added in DMSO solution, and the cells were incubated for a further period of 6 and 24 h. Two hours prior to harvesting, the cells were arrested in C-mitosis by addition of 10 ug/mL Colcemid and harvested. The suspended cells were collected by centrifugation at 400 *g* and submitted to hypotonic treatment (75 mM KCl, 37°C, 20 min). After further centrifugation, the cells were fixed by dropwise addition of ice-cold, freshly prepared methanol/acetic acid fixative (3:1, v/v) under contiuous shaking. The fixed cells were left in the refrigerator for at least 30 min, but preferably overnight; they were then spun down and fresh fixative was added; after further centrifugation, the cells were taken up in a few drops of fresh fixative and dropped on ice-cold, wet slides, where the metaphases were spread by vigorous blowing and cautious flaming. Chromosomes were stained in orcein (2% in 60% acetic acid) for 10 min. The slides were cleared by dipping them twice briefly into absolute ethanol and by treating them in two changes of xylene before mounting. Althoug the slides were not coded, every precaution was taken to prevent bias.

Metabolic activation was not applied. Resorcinol diglycidyl ether (>98% purity) was tested at concentrations of 0, 2.5, 8, 25 µg/ml. A positive control was not used, and details on the negative control was not specified.

Table 3.8.1.6-1. In vitro chromosomal aberrations in chinese hamster ovary cells induced by resorcinol diglycidyl ether (Seiler 1984).

	Treatment time (h)	**Conc. (µg/ml)**	**Metaphases scored**	**No (%) aberrant metaphases**	**Number of**		**remarks**
					breaks	**Trans-locations**	
control		0	100	2 (2)	3		
Resorcinol diglycidyl ether							
	6	2.5	100	8 (8)	11		
		8	33	8 (24)	10		*P*<0.01
		25	25	11 (44)	16		*P*<0.01
	24	2.5	100	9 (9)	9	4	
		8	50	24 (48) [a]	18	7	*P*<0.01
		25	15	14 (93) [a]	14	1	*P*<0.01

[a] Methaphases with multiple aberrations. Not presented in this table, but used for the total frequency calculations, were diverse aberrations like rings, acebtric fragments, dicentrics.

Outcome: Resorcinol diglycidyl ether induced chromosal aberration (Table 3.8.1.6-1), at the highest tested concentration upon 24 h exposure no cell was virtually undamaged. Chromosomal damage ranged from single chromatid breaks, through multiple aberrations, to completely pulverized metaphases.

3.8.1.7 Study 7: Gulati (1989)

Gulati et al. (1989) tested the sister chromatid exchange (SCE) potential of resorcinol diglycidyl ether in Chinese hamster ovary cells.

Briefly, approximately 24h prior to cell treatment, 1 x 10^6 Chinese hamster ovary cells were seeded per 75 cm^2 flask. A culture was established for each dose both with and without metabolic activation (S9). A liver fraction (S9) prepared from Aroclor 1254-induced male Sprague-Dawley rats was used to provide exogenous metabolic activation. The final concentrations of the S9 fraction, NADP, and isocitric acid were 0.02 ml, 2.4 mg, and 4.5 mg, respectively per ml culture medium. For assays without metabolic activation, the medium was replaced with fresh medium immediately before treatment with resorcinol diglycidyl ether. Cells were treated with test or control substances for 2 h to allow interaction with cells before the addition of bromodeoxyuridine (BrdUrd). BrdUrd was then added (final concentration 10 µM), and incubation was continued for an additional 24 h. The medium was removed and fresh medium containing 10 µM BrdUrd and colcemid was added and incubation was continued for 2-3 h. For assays with metabolic activation, the cells were rinsed twice with phosphate buffered saline (PBS), after which culture medium without FBS was added. Cells were incubated for 2 h in the presence of the test or control substance and the S9 reaction mixture. FBS was omitted to avoid the binding of serum proteins to short-lived, highly reactive intermediates. After the 2h exposure period, cells were washed twice with PBS, and then complete medium containing 10% FBS and 10 µM BrdUrd was added. Cells were incubated for an additional 26 h, with colcemid present for the final 2-3 h of incubation. Two to three hours after addition of colcemid, cells were harvested by mitotic shake-off. Prior harvesting, the percent confluency in each flask was estimated using a wide-field microscope. Harvested cells were treated for about 3 min at room temperature with hypotonic KCl (75 mM), washed with fixative (3:1 methanol: glacial acetic acid, v/v), dropped onto slied, and air dried. Staining for the detection of SCE was accomplished by a modified fluorescence plus Giemsa technique. Fifty second-division metaphase cells were scored per dose for the incidence of SCE. The number of chromosomes in each cell was also recorded. Any cell that had fewer than 19 or more than 23 chromosomes was excluded. All slides except for the high-dose positive controls were coded. Positive results in initial tests were confirmed by additional tests. If both –S9 and +S9 studies gave a positive response and required confirmation, they were done sequentially (-S9 first). If the –S9 repeat was positive, the repeat +S9 study was not always performed.

Resorcinol diglycidyl ether (>87.9% purity) was tested in concentrations of 0, 0.05, 0.16, 0.5, (1.6 only in 1st test) µg/ml (-S9) adn 0, 0.5, 1.6, 5, 16 µg/ml (+S9). As solvent, DMSO was used. Positive controls were mitomycin C (-S9) and cyclophosphamide (+S9).

Outcome: Treatment of cultured CHO cells with resorcinol diglycidyl ether produced highly significant increases in sister chromatid exchanges, with and without metabolic activation. The number of SCE/cell (* indicates statistical significance):

- 7.7, 9.7*, 10*, 30*, 71* (-S9, 1st test)
- 9.1, 8.4, 21*, 49* (-S9, 2nd test)
- 9.6, 9.8, 10, 13*, 51* (+S9, 1st test)
- 9.4, 8.5, 9.9, 14*, 39* (+S9, 2nd test)

3.8.1.8 Study 8: Seiler (1984b)

The alkylating potency of resorcinol diglycidyl ether was measured in the 4-(4-nitrobenzyl)pyridine (NBP) assay according to Friedman and Boger (1961).

A positive control was not used, and details on the negative control was not specified. Resorcinol diglycidyl ether was tested at concentrations of 12.5, 25, 50 and 100 µg. The optical density at 540 nm (as measured against a negative control) was 0.23, 0.55, 1.17 and 2.18 respectively Showing the alkylating potential of resorcinol diglycidyl ether in vitro.

3.8.2 Animal data

3.8.2.1 Study 1: Shelby et al. (1993)

Shelby et al. (1993) performed an in vivo mouse bone marrow micronucleus. Groups of 5-7 male B6C3F1 mice (age 9-14 wk; weight 25-33 g) were administered resorcinol diglycidyl ether (in corn oil) by intraperitoneal injection on three consecutive days. The study was performed in triplicate, (15.2, 30.4, 60.8 mg/kg resorcinol diglycidyl ether (first and second test), 30.4, 60.8, 91.2 mg/kg resorcinol diglycidyl ether (third test)), dimethylbenzanthracene was used as positive control. Mice were euthanized with CO_2 24 h after the third treatment. Bone marrow smears (two slides per mouse) were prepared, fixed in absolute methanol, and stained with acridine orange. For each animal, slides were evaluated at 1000x magnification for the number of micronucleated-PCE among 2000 PCE and for the percentage of PCE among 200 erythrocytes. The %PCE were analysed by an analysis of variance test based on pooled data. Pairwise comparisons between each group and the concurrent solvent control group was by an unadjusted one-tailed Pearson chisquared test which incorporated the calculated variance inflation factor for the study.

Outcome: The test was positive to 60.8 mg/kg bw with trend P=0.038. Repeat tests to 60.8 mg/kg bw and 91.2 mg/kg bw were both negative and the overall results was concluded to be negative. All animals survived and no toxicity to PCE was observed..

3.8.2.2 Study 2: Shelby et al. (1993)

Shelby et al. (1993) stated that “resorcinol diglycidyl ether was highly effective at inducing chromosomal aberrations in mouse bone marrow cells following single exposure up to 300 mg/kg bw”. Therefore, they conducted as a follow-up to the experiments described in 3.8.2.1 an additional micronucleus study under the same conditions as the before except that they used a single-exposure. The animals were treated with 90, 180, 270 mg/kg bw resorcinol diglycidyl ether.

Outcome: A significant dose-related increase in micronucleated-PCE was observed at doses of 90, 180, and 270 mg/kg bw. The study authors considered that due to the toxicity characteristics of resorcinol diglycidyl ether, a three-exposure protocol does not permit use of a sufficiently high exposure to induce observable genetic toxicity.

3.8.2.3 Study 3: Seiler (1984b)

An in vivo micronucleus test was performed in ICR mice. Male and female mice (4 animals/dose) were given resorcinol diglycidyl ether (>98% purity) orally, dissolved in polyethylene glycol (PEG400), in doses up to acutely toxic levels (300 and 800 mg/kg bw). 24h after this single exposure, mice were sacrificed and bone marrow cells were flushed out into foetal calf serum. In the case of a negative outcome of the test, a second assay was performed with fixation times of 24, 48 and 72 h. After centrifugation at 400 *g*, the cells were spread onto slides, air-dried and stained with May-Grunewald Giemsa. The slides were coded and analysed by two individuals separately.

Outcome: Resorcinol diglycidyl ether was found to be inactive with respect to induction of micronuclei *in vivo*.

Table 3.8.2.3-1. Induction of micronucleated polychromatic erythrocytes in the bone marrow of mice treated with resorcinol diglycidyl ether

	Frequency (and standard deviation) of micronucleated erythrocytes (per thousand polychromatic erythrocytes)	
Fixation time (h)	**Dose levels (mg/kg bw)**	
	300	**600**
24	0.0 (1.5)	0.0 (1.9)
38		0.9 (1.3)
72		0.4 (0.6)

The spontaneous frequency of micronucleated polychromatic erythrocytes (3-5 per thousand) has already been subtracted.

3.8.3 Human data

N/A

3.8.4 Other data

N/A

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 Study 1: NTP (1986); Krishna-Murthy et al. (1990) – rat

Groups of 50 F344/N rats of each sex received resorcinol diglycidyl ether (technical grade; 81% purity, identity of impurities not characterized) in corn oil by gavage 5 days per week for 103 weeks at doses of 0, 25 or 50 mg/kg bw/d (NTP 1986; Krishna-Murthy et al., 1990). Animals were housed five per cage and diets and tap water were available ad libitum. All animals were observed twice daily for signs of morbidity or mortality. Clinical signs were recorded monthly. Body weights by cage were recorded every week for the first 12 weeks and montly thereafter. Moribund animals and animals that survived to the end of the study were killed with carbon dioxide and necropsies were killed.

Macro- and microscopic evaluation: Examinations for grossly visible lesions were performed on major tissues and organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following were examined microscopically: tissue masses, abnormal lymph nodes, skin, mandibular or mesenteric lymph nodes, mammary gland, salivary gland, sternebrae, bone marrow, thymus, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, small intestine, colon, liver, pancreas, spleen, kidneys, adrenals, urinary bladder, prostate/ testes or ovaries/ uterus, brain, and pituitary. Necropsies were performed on all animals unless precluded in whole or in part by autolysis or cannibalization.

Data-analysis: Fisher's exact test for pairwise comparison, Cochran-Armitage linear trend test for dose response trends. Two methods adjusting for intercurrent mortality using combining contingency tables by Mantel and Haenszel (1959) (life table test & incidental tumour test)

Results: A summary of the main results in presented in Table 3.9.1.1-1.

The survival of male and female rats was significantly reduced ($p<0.001$) compared to controls, and the high dose group of each sex had significantly lower survival ($p<0.001$) than the low dose group (Figure 3.9.1.1-1). At the end of the study (104-105 weeks), 42, 5 and 0 male and 37, 16 and 1 female rats of the control, low dose and high dose groups, respectively, had survived. Most of the early deaths not related to tumour induction were attributable to bronchopneumonia.

Body weights were decreased in all treated rats and more so in rats in the high-dose group (Table 3.9.1.1-2).

Starting from week 30, mean body weights of high dose rats of each sex were lower than those of the controls. Except for weeks 80 to 100, mean body weights of low dose males and females were comparable with those of the controls. Wheezing and respiratory distress were the only compound-related clinical signs observed.

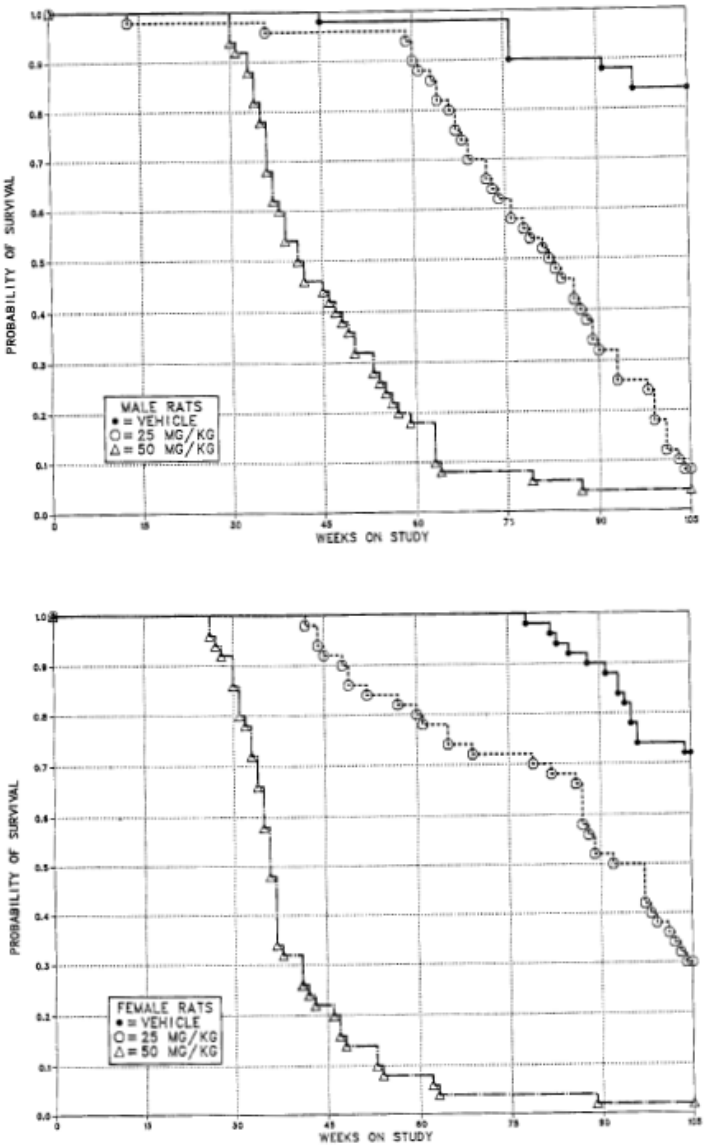

Figure 3.9.1.1-1. Survival curves for F344/N rats administered resorcinol diglycidyl ether in corn oil by gavage for 2 years (NTP 1986; Krishna-Murthy et al., 1990).

Table 3.9.1.1-1. Summary of main results observed in F344/N rats, which were given resorcinol diglycidyl ether by gavage for 2 years (NTP 1986; Krishna-Murthy et al., 1990).

Dose (mg/kg bw/day)	**0**		**25**		**50**	
	m	**f**	**m**	**f**	**m**	**f**
Mortality	8/50	13/50	45/50	34/50	50/50	49/50
Body weight			d	d	d	d
Clinical signs						
Wheezing and respiratory distress			i	i	i	i
Pathology						
Non-neoplastic lesions						
Lungs						
Bronchopneumonia	2/50	0/50	17/49	10/50	26/50	17/50
Forestomach						
Basal cell hyperplasia	1/50 (2%)	2/49 (4%)	16/50 (32%)	12/50 (24%)	34/50 (68%)	33/50 (66%)
Hyperkeratosis	1/50 (2%)	1/49 (2%)	12/50 (24%)	12/50 (24%)	43/50 (86%)	48/50 (96%)
Neoplastic lesions						
Forestomach						
Squamous cell papilloma	0/50	0/49	17/50 (34%) P<0.001	7/50 (14%) P=0.002	6/49 (12%) P=0.012	1/50 (2%) P=0.125
Squamous cell carcinoma	0/50	0/49	38/50 (76%) P<0.001	34/50 (68%) P=0.001	4/49 (8%) P=0.056	3/50 (6%) P=0.125
Adenocarcinoma	0/50	0/49	1/50	0/50	0/50	0/50
Leiomyosarcoma	0/50	0/49	0/50	1/50	0/50	0/50
Carcinosarcoma	0/50	0/49	0/50	1/50	0/50	0/50

d= decreased versus control
i= increased versus control
Fischer exact test

Table 3.9.1.1-2. Mean body weights of F344/N rats administered resorcinol diglycidyl ether in corn oil by gavage for two years (NTP 1986; Krishna-Murthy et al., 1990).

Weeks on study	**Body weight (g) #**		
	Vehicle control	*Low dose (25 mg/kg bw/d)*	*High dose (50 mg/kg bw/d)*
Male			
0	179	171 (95.5)	174 (97.2)
24	387	376 (97.2)	362 (93.5)
52	468	452 (96.6)	426 (91.0)
80	462	440 (95.2)	411 (89.0)
100	461	417 (90.5)	-*
104	449	378 (84.2)	-*
Female			
0	127	128 (100.8)	129 (101.6)
24	223	219 (98.2)	217 (97.3)
52	265	259 (97.7)	248 (93.6)
80	304	292 (96.1)	275 (90.5)
100	336	291 (86.6)	270 (80.4)
104	337	288 (85.5)	268 (79.5)

values in brackets: percentage of control animals
* All animals dead on this time point

Treatment-related lesions were primarily observed in the forestomach of low and high dose rats of both sexes. Resorcinol diglycidyl ether produced hyperkeratosis, hyperplasia, and neoplasms of the squamous epithelium of the forestomach. The squamous epithelium of the esophagus and nasopharynx was

hyperkeratotic in some rats, but no tumors were found. Postmortem examination of the stomachs revealed numerous small rough nodules on the nonglandular mucosa that progressed in some animals to form large, white, fungiform masses which were occasionally ulcerated. The larger lesions involved adjacent tissues such as the spleen, pancreas, and lymph nodes. Histologically, the thickened mucosa showed intense hyperkeratosis that was usually accompanied by hyperplasia of the basal layers. Small nodules, diagnosed as squamous papillomas, were characterized by projections of hyperkeratotic epithelium supported by a fibrovascular core. These changes appeared to be identical to those found in the 13-week study (see section 3.12.1.3). In the larger masses, the basal cells developed hyperchromatism, pleomorphism, and parachromatin clearing, all signs of malignancy. The masses grew downward through the basement membrane into and through the muscularis mucosa in irregular strands and clumps, and keratin pearls were often produced. Both normal and abnormal mitotic figures were frequently observed. These lesions were diagnosed as squa mous cell carcinomas. Metastases from these tumors were found in 14 low dose males, 1 high dose male, and 5 low dose females. Metastatic tumors were found in the regional lymph nodes, pancreas, liver, spleen, lungs, and brain.

Low and high dose male and female rats had statistically significantly increased incidences of squamous cell papilloma and squamous cell carcinoma of the forestomach. Of the male rats 0/50, 17/50 (34%) and 6/49 (12%) developed squamous cell papilloma in the control, low, and high dose group, respectively. When these numbers were adjusted for intercurrent mortality the incidences were 0, 40.9 and 33.5%, respectively. The incidence of squamous cell carcinoma in male rats was 0/50, 38/50 (76%) and 4/49 (8%), respectively (adjusted incidence: 0, 100, 100%). Of the female rats 0/49, 7/50 (14%) and 1/50 (2%) developed squamous cell papilloma in the control, low, and high dose group, respectively (adjusted incidence: 0, 24.2, 14.3%). The incidence of squamous cell carcinoma in female rats was 0/49, 34/50 (68%) and 3/50 (6%), respectively (adjusted incidence: 0, 97, 100%). The lower number of papilloma and carcinoma in the high dose group compared to the low dose group probably resulted from the increased number of early deaths at the high dose. A detailed overview on these tumour incidences can be found in table 3.9.1.1-3.

Table 3.9.1.1-3. Incidences of neoplasms of the stomach in male and female rats administered resorcinol diglycidyl ether in corn oil by gavage for two years (NTP, 1986; Krishna-Murthy, 1990).

	Dose resorcinol diglycidyl ether (mg/kg bw/d)					
	0		**25**		**50**	
	m	f	m	f	m	f
Squamous cell papilloma						
Overall incidence	0/50 (0%)	0/49 (0%)	17/50 (34%)	7/50 (14%)	6/49 (12%)	1/50 (2%)
Adjusted incidence[(a)]	0.0%	0.0%	40.9%	24.2%	33.5 %	14.3%
Terminal incidence	0/42 (0%)	0/36 (0%)	0/5 (0%)	1/16 (6%)	0/0 (0%)	0/1 (0%)
Life table test	P<0.001	P<0.001	P<0.001	P=0.002	P<0.001	P=0.125
Cochran-Armitage trend test	P=0.058	P=0.421				
Fischer Exact test			P<0.001	P=0.007	P=0.012	P=0.505
Squamous cell carcinoma						
Overall incidence	0/50 (0%)	0/49 (0%)	38/50 (76%)	34/50 (68%)	4/49 (8%)	3/50 (6%)
Adjusted incidence[(a)]	0.0%	0.0 %	100%	97.0%	100%	100.0%
Terminal incidence	0/42 (0%)	0/36 (0%)	5/5 (100%)	15/16 (94%)	0/0 (0%)	1/1 (100%)
Life table test	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Cochran-Armitage trend test	P=0.199	P=0.300				
Fischer Exact test			P<0.001	P=0.001	P=0.056	P=0.125

(a) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality

According to the study report it was stated that the statistical analyses and interpretation of the tumor incidence data for rats in this study were complicated by the marked reduction in survival in dosed male and female rats when compared with that of the controls. No control animals were killed when the dosed animals were dying. Consequently, the incidental tumor test is of little or no value, since there is little overlapping of survival in dosed and control groups. This test was not performed for rats. In addition, the results of the life table and "unadjusted" analyses (i.e., the Fisher exact and Cochran- Armitage tests) were frequently contradictory. That is, the life table analysis often indicated a significant positive trend while the unadjusted analysis indicated a significant negative trend. These results were produced because life table analysis is sensitive to the time at which animals die with tumors. Thus, for these data life table analyses give more

emphasis to tumors in the dosed groups (which generally occurred in animals dying early in the study) than to tumors in the controls (which generally occurred later in the study, when few dosed animals were alive for comparative purposes). Conversely, the unadjusted analysis compares only the overall tumor rates. These incidences are frequently lower in the dosed groups than in the controls because of the early deaths in the dosed groups. Because of these problems, evidence of a positive effect by both life table and unadjusted analyses was considered necessary before an increase in tumor incidence was regarded as being related to chemical administration. These criteria would not apply when neoplasms are clearly recognized as the cause of death; life table analysis would be appropriate in this instance.

Metastases were observed in 20 out of 144 rats and the sites of metastases were brain, liver, lung, lymph nodes, pancreas and spleen. Other neoplasms of the stomach that occurred in a few rats were: adenocarcinoma of the glandular stomach (25 mg/kg bw/d, 1 male), basal cell carcinoma (12 mg/kg bw/d, 1 female), leiomyosarcoma (25 mg/kg bw/d, 1 female), and carcinosarcoma (25 mg/kg bw/d, 1 female). It was stated by the authors that the possibility that the leiomyosarcoma and carcinosarcoma may be poorly differentiated squamous cell carcinoma should be considered (Krishna-Murthy et al., 1990).

Bronchopneumonia was the most frequent cause of early death in rats and was characterized by patches of polymorphonuclear leukocytes in the alveoli of the lung, especially near the bronchi. Polymorphonuclear leukocytes also occurred in masses within bronchi and in the bronchial epithelium. In some rats, pulmonary vessels were dilated and showed perivascular edema. Microbiological examination were not conducted on these rats.

In this primary study, several other types of neoplasms occurred in rats with overall incidences that were lower in the dosed groups than in the controls; these included adrenal pheochromocytoma, leukemia, pituitary adenoma, and thyroid C-cell tumors in males and females; lung adenoma, pancreatic islet cell tumors, and interstitial cell tumors of the testes in males; and mammary glandfibroadenomas and uterine tumors in females. None of these decreases were statistically significant when life table analyses were used, and they appeared to be related to the reduced survival observed in the dosed groups relative to those in the controls.

Supplemental study

Because of high early mortality at the high dose (50 mg/kg bw/d), a supplemental study exposing rats to 0 and 12 mg resorcinol diglycidyl ether (technical grade; 81% purity, identity of impurities not characterized)/kg body weight was performed 12 months after the start of the primary study (NTP 1986; Krishna-Murthy et al., 1990). Except for the dose, the protocol of this study was identical to that of the original study.

Results supplemental study: A summary of the main results in presented in Table 3.9.1.1-4.

Survival of the male dosed rats was significantly reduced (p=0.003) compared to controls. Survival of dosed and control female rats did not differ significantly (Figure 3.9.1.1-2). The numbers of rats that lived to the end of the study were 39 control and 23 dosed males and 39 control and 35 dosed females. The reduced survival in males was probably due to the increase in squamous cell carcinomas as the incidence of intercurrent mortality without such tumours was comparable between the treated group ((27-19)/50) and the controls (11/50). Body weight gain was not affected in the dosed rats (Table 3.9.1.1-5).

The incidence of hyperkeratosis and basal cell hyperplasia in the forestomach was markedly increased in dosed males and females. Regarding tumour development, 32% of the male rats and 38% of the female rats developed squamous cell papilloma (adjusted incidence 51.7 and 48.4%). Squamous cell carcinoma was observed in 78% and 54% (adjusted incidence 92.8 and 64%) of the male and female rats, respectively. These tumours were not observed in the control rats of either sex. A detailed overview on these tumour incidences can be found in table 3.9.1.1-6.

In this supplemental study, neurofibrosarcomas were observed at an increased incidence in dosed male rats (control, 0/50; dosed, 3/50), but the increase was not statistically significant. The incidence of C-cell tumors of the thyroid was significantly reduced in the dosed males compared to controls (control, 11/50; dosed, 3/50; $P<0.03$, incidental tumor and Fisher exact tests). In female rats, there was a statistically significant decrease ($P<0.05$) in the incidence of pheochromocytomas of the adrenal medulla in the dosed group (control, 5/50; dosed, 0/50).

Table 3.9.1.1-4. Summary of main results observed in F344/N rats, which were given resorcinol diglycidyl ether by gavage for 2 years: supplemental study (NTP 1986; Krishna-Murthy et al., 1990).

Dose (mg/kg bw/day)	**0**		**12**	
	m	**f**	**m**	**f**
Mortality	11/50	11/50	27/50	15/50
Body weight			No treatment-related effect	
Clinical signs			No treatment-related effect	
Pathology				
Non-neoplastic lesions				
Forestomach				
Basal cell hyperplasia	6/50 (12%)	3/50 (6%)	37/50 (74%)	45/50 (90%)
Hyperkeratosis	0/50 (0%)	0/50 (0%)	38/50 (76%)	46/50 (92%)
Neoplastic lesions				
Forestomach				
Squamous cell papilloma	0/50	0/50	16/50 (32%) P<0.001	19/50 (38%) P<0.001
Squamous cell carcinoma	0/50	0/50	39/50 (78%) P<0.001	27/50 (54%) P=0.001
Basal cell carcinoma	0/50	0/50	0/50	1/50

d= decreased versus control
i= increased versus control
Fischer exact test

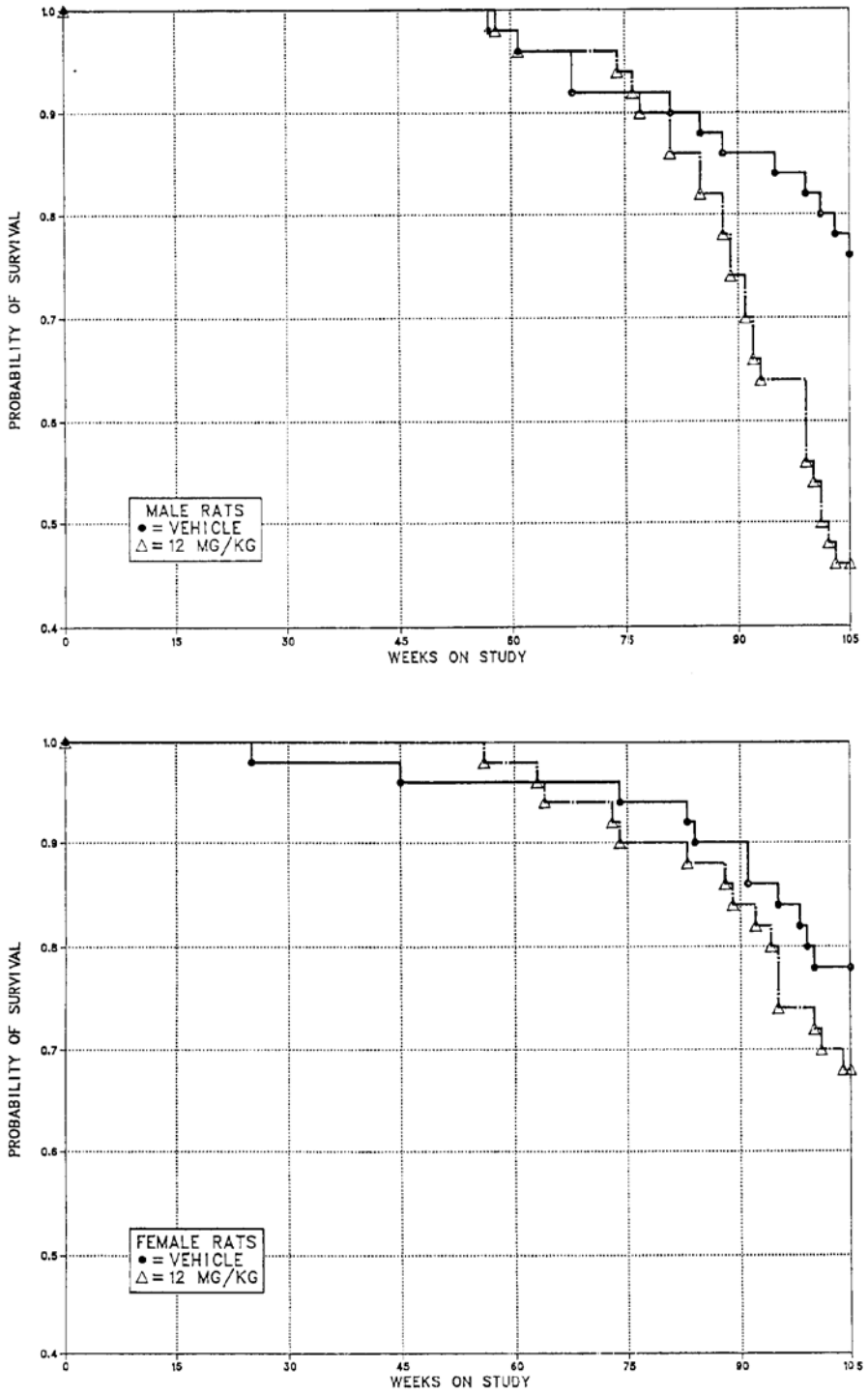

Figure 3.9.1.1-2. Survival curves for F344/N rats administered resorcinol diglycidyl ether in corn oil by gavage for 2 years; supplemental study (NTP 1986; Krishna-Murthy et al., 1990).

Table 3.9.1.1-5. Mean body weights of F344/N rats administered resorcinol diglycidyl ether in corn oil by gavage for two years: supplemental study (NTP 1986; Krishna-Murthy et al., 1990).

	Body weight (g) #	
	0 mg/kg bw/d	*12 mg/kg bw/d)*
Male		
0	181	184 (101.7)
24	378	383 (101.3)
52	456	463 (101.5)
80	480	472 (98.3)
104	450	434 (96.4)
Female		
0	131	130 (99.2)
24	211	205 (97.2)
52	263	261 (99.2)
80	310	304 (98.1)
104	323	317 (98.1)

values in brackets: percentage of control animals

Table 3.9.1.1-6. Incidences of neoplasms of the stomach in male and female rats administered resorcinol diglycidyl ether in corn oil by gavage for two years: supplemental study (NTP, 1986; Krishna-Murthy, 1990).

	Dose resorcinol diglycidyl ether (mg/kg bw/d)			
	0		**12**	
	m	f	m	f
Squamous cell papilloma				
Overall incidence	0/50 (0%)	0/50 (0%)	16/50 (32%)	19/50 (38%)
Adjusted incidence[(a)]	0.0%	0.0%	51.7%	48.4%
Terminal incidence	0/39 (0%)	0/39 (0%)	10/23 (43%)	15/35 (43%)
Life table test			P<0.001	P<0.001
Incidental tumour test			P<0.001	P<0.001
Fischer Exact test			P<0.001	P<0.001
Squamous cell carcinoma				
Overall incidence	0/50 (0%)	0/50 (0%)	39/50 (78%)	27/50 (54%)
Adjusted incidence[(a)]	0.0%	0.0 %	92.8%	64.0%
Terminal incidence	0/39 (0%)	0/39 (0%)	20/23 (87%)	20/35 (57%)
Life table test			P<0.001	P<0.001
Incidental tumour test			P<0.001	P<0.001
Fischer Exact test			P<0.001	P<0.001

(a) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality

3.9.1.2 Study 2: NTP, 1986 – mouse

Groups of 50 B6C3F1 mice of each sex were administered 0, 50 or 100 mg resorcinol diglycidyl ether (technical grade; 81% purity, identity impurities not characterized)/kg bw/d in corn oil via gavage 5 days per week for 103 weeks (NTP 1986; Krishna-Murthy et al., 1990). Animals were housed five per cage and diets and tap water were available ad libitum. All animals were observed twice daily for signs of morbidity or mortality. Clinical signs were recorded monthly. Body weights by cage were recorded every week for the first 12 weeks and montly thereafter. Moribund animals and animals that survived to the end of the study were killed with carbon dioxide and necropsies were killed.

Macro- and microscopic evaluation:

Examinations for grossly visible lesions were performed on major tissues and organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following were examined microscopically: tissue masses, abnormal lymph nodes, skin, mandibular or mesenteric lymph nodes, mammary gland, salivary gland, sternebrae, bone marrow, thymus, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, small intestine, colon, liver, gallbladder, pancreas, spleen, kidneys, adrenals, urinary bladder, prostate/ testes or ovaries/ uterus, brain, and pituitary.

Necropsies were performed on all animals unless precluded in whole or in part by autolysis or cannibalization.

Data-analyis: see rat study (section 3.9.1.1)

Results: A summary of the main results in presented in Table 3.9.1.2-1.

Mean body weights of high dose female mice were lower than those of the controls after week 20 of the study. Mean body weights of high and low dose male mice and of low dose female mice were comparable with those of the controls (Table 3.9.1.2-2).

No compound-related clinical signs were observed.

No significant differences in survival were observed between the dosed and control groups, but survival was only 40, 26, and 20% in control, low dose and high dose female mice, respectively (figure 3.9.1.2-1). The major cause of death in dosed female mice was a necrosuppurative lesion of the ovary which spread to other areas of the abdominal cavity.

Table 3.9.1.2-1. Summary of main results observed in B6C3F1 mice, which were given resorcinol diglycidyl ether by gavage for 2 years (NTP 1986; Krishna-Murthy et al., 1990).

Dose (mg/kg bw/day)	**0**		**50**		**100**	
	m	**f**	**m**	**f**	**m**	**f**
Mortality	20/50	30/50	24/50	37/50	16/50	40/50
Body weight						d
Clinical signs			No treatment-related effects		No treatment-related effects	
Pathology						
Non-neoplastic lesions						
Forestomach						
Hyperplasia	1/47	3/47	30/49	25/49	37/50	26/49
Hyperkeratosis	3/47	11/47	40/49	31/49	42/50	46/49
Neoplastic lesions						
Forestomach						
Squamous cell papilloma or papillomatosis*	0/47	0/47	4/49 (8%) P=0.064	5/49 (10%) P=0.031	10/50 (20%) P=0.001	10/49 (20%) P=0.001
Squamous cell carcinoma	0/47	0/47	14/49 (29%) P<0.001	12/49 (24%) P<0.001	25/50 (50%) P<0.001	23/49 (47%) P<0.001
Adenocarcinoma	0/47	0/47	0/49	0/49	1/49	0/49
Liver						
Hepatocellular adenoma	7/48 (15%)	3/48 (6%)	7/50 (14%)	0/50 (0%) P=0.114	5/50 (10%)	5/49 (10%) P=0.369
Hepatocellular carcinoma	7/48 (15%)	0/48 (0%)	11/50 (22%)	1/50 (2%) P=0.510	6/50 (12%)	3/49 (6%) P=0.073
Hepatocellular carcinoma and adenoma combined	14/48 (29%)	3/48 (6%)	18/50 (36%)	1/50 (2%) P=0.294	11/50 (22%)	7/49 (14%) P=0.167

d= decreased versus control
i= increased versus control
Fischer exact test
* Papillomatosis is a term used by the contractor pathologist to describe multiple papillomas in the stomach of a single animal

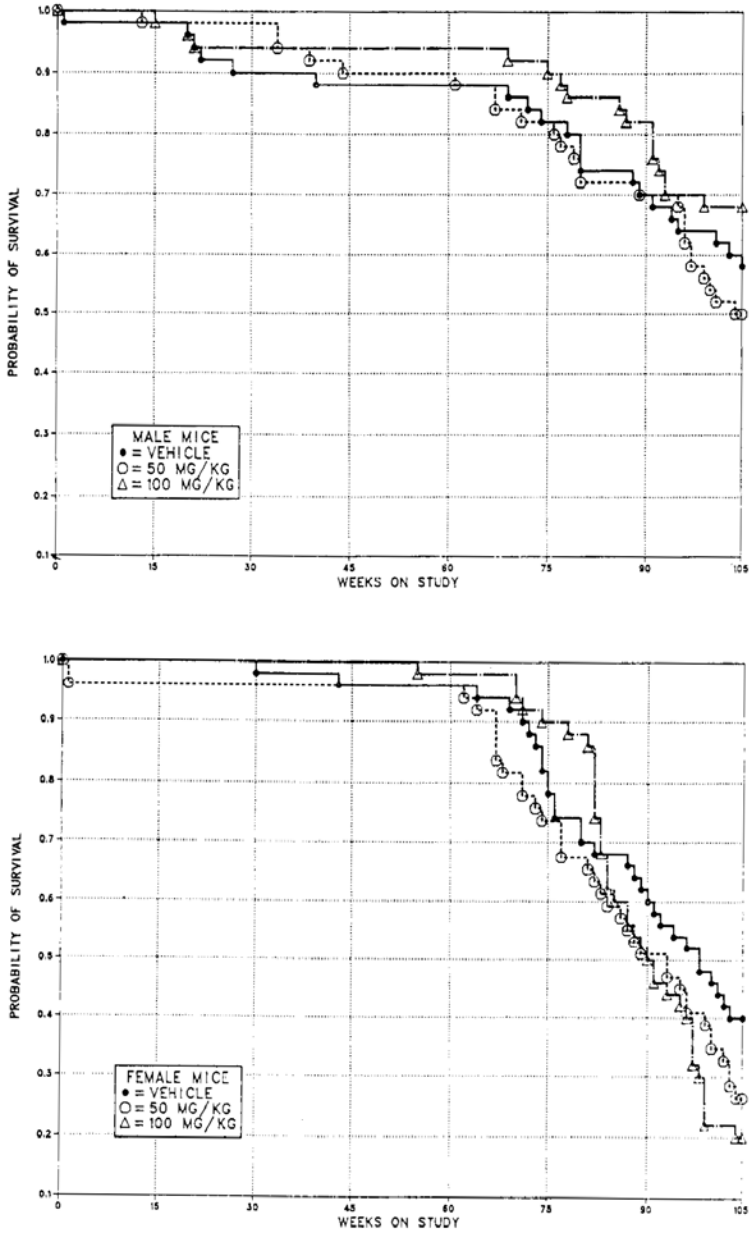

Figure 3.9.1.2-1. Survival curves for mice administered resorcinol diglycidyl ether in corn oil by gavage for 2 years (NTP 1986; Krishna-Murthy et al., 1990).

Table 3.9.1.2-2. Mean body weights of B6C3F1 mice administered resorcinol diglycidyl ether in corn oil by gavage for two years (NTP 1986; Krishna-Murthy et al., 1990).

Weeks on study	**Body weight (g) #**		
	Vehicle control	*Low dose (50 mg/kg bw/d)*	*High dose (100 mg/kg bw/d)*
Male			
0	23	24 (104.3)	24 (104.3)
24	38	38 (100.0)	39 (102.6)
52	41	43 (104.9)	43 (104.9)
80	41	43 (104.9)	41 (100.0)
104	39	38 (97.4)	38 (97.4)

Weeks on study	**Body weight (g) #**		
	Vehicle control	*Low dose (50 mg/kg bw/d)*	*High dose (100 mg/kg bw/d)*
Female			
0	20	20 (100.0)	20 (100.0)
24	32	32 (100.0)	30 (93.8)
52	40	39 (97.5)	44 (110.0)
80	43	42 (97.7)	36 (83.7)
104	43	41 (95.3)	34 (79.1)

values in brackets: percentage of control animals

Treatment-related lesions were primarily observed in the forestomach of low and high dose rats of both sexes. The incidence of hyperkeratosis and epithelial cell hyperplasia in the forestomach was markedly increased in low- and high-dose mice of both sexes.

Squamous cell papillomas and carcinomas and papillomatosis of the forestomach occurred in male and female mice with statistically significant positive trends and the incidences in the high dose groups were significantly higher than those in the controls. The squamous cell papillomas were papillary growths of the epithelium and were supported by a narrow or broad fibrovascular stalk. They were covered by markedly thickened epithelium which was often heavily keratinized. Multiple lesions in the stomach of a single animal were referred to as papillomatosis. Squamous cell carcinomas were characterized by infiltrative growth into the submucosa and muscularis. The component cells varied in size and shape and many had indistinct margins. The cytoplasm was more eosinophilic than normal and, in some cells in the superficial layers, it contained keratohyalin granules. Nuclei were enlarged and contained coarse or stippled chromatin and one or two prominent nucleoli. Mitotic figures were present but not numerous. Keratin pearls were present in many of the carcinomas of different sizes. The lumina of the large pearls were filled with desquamated material, inflammatory cells, and necrotic debris. Nonkeratinizing squamous cell carcinomas were seen in the forestomach of a few mice. Areas of necrosis and hemorrhage were common in the large tumors. The morphology of the gastric neoplasms in mice was comparable to that obtained in rats (see section 3.9.1.1).

Of the male mice 0/47, 4/49 (8%) and 10/50 (20%) (adjusted incidence: 0, 14, 29.4%) developed squamous cell papilloma or papillomatosi in the control, low, and high dose group, respectively. The incidence of squamous cell carcinoma in male mice was 0/47, 14/49 (29%) and 25/50 (50%), respectively (adjusted incidence: 0, 40.7, 55.5%). Of the female mice 0/49, 5/49 (10%) and 10/49 (20%) developed squamous cell papilloma or papillomatosis, respectively (adjusted incidence: 0, 33.4, 73.1%). The incidence of squamous cell carcinoma in female mice was 0/47, 12/49 (24%) and 23/49 (47%), respectively (adjusted incidence: 0, 53.3, 70.5%). The carcinoma had metastasized in 25 out of 74 mice (50 mg/kg bw/d, 4 male, 1 female; 100 mg/kg bw/d, 10 male and 10 female) and the sites of metastases were adrenal glands, brain, heart, kidney, liver, lung, lymph nodes and spleen. Adenocarcinoma of the glandular stomach was diagnosed in one male mouse in the 100 mg/kg bw/d group.

While pairwise comparisons were not significant ($P>0.05$), a positive trend was observed in the incidence of female mice with hepatocellular carcinoma. The incidences of hepatocellular carcinoma and of hepatocellular adenoma and carcinoma combined were statistically significantly increased in female mice in the high dose group compared to controls (respectively, $p=0.041$ and 0.030 by life-table analysis). The incidences of females with either adenomas or carcinomas had a significant positive trend; and the pairwise comparison between the high dose group and the controls was significant by the life table test. No positive trend or statistically significant increase in hepatocellular carcinoma was noticed for male mice. These liver tumours were probably not related to the administration of the test substance because their incidence in females dosed with the test substance (6% for carcinoma, 14% for combined adenoma/carcinoma) was lower than that in historical controls at the same research programm (upper level 8% for carcinoma and 14% for combined adenoma/carcinoma). See table 3.9.1.2-3 for historical control data.

The ovaries were enlarged and filled with a viscous yellow exudate in 17/50 control, 12/50 low dose, and 15/50 high dose females. Ovarian tissue was not macroscopically recognizable in many of these masses. Microscopically, a mantle of neutrophils, macrophages, and fibrosis surrounded the multiple abscesses.

Extensive adhesions were present between the mass and the omentum. Neutrophils, lymphocytes, and plasma cells were present in the adja cent adipose tissue. Fibrinoid exudate was disseminated both in the abdominal and thoracic cavities. Overall, necrotizing inflammation was found in the abdominal cavity, ovary, uterus, or multiple organs in 18/30 vehicle control, 18/36 low dose, and 16/40 high dose females that died before the end of the study. Although microbiologic examinations were not performed on mice in this study, Klebsiella oxyroca has been isolated from mice that had similar lesions in other studies performed at the same laboratory.

Mineralization was found in the kidneys of 8/50 control males, 18/50 low dose males, and 30/50 high dose males. The mineralization was minimal and the distribution was multifocal, being located primarily in the cortex. The foci were small, ranging from the size of one or two renal tubular epithelial cells to the size of a tubule.

Malignant lymphocytic lymphomas, malignant lymphomas of mixed type, and malignant lymphomas of all types occurred in female mice with negative dose-related trends, i.e. the incidence of all types of malignant lymphomas was significantly lower in the high dose group than in the controls. In addition, fibroma, fibrosarcoma, or carcoma occurred in male mice with a statistically significant negative trend.

Table 3.9.1.2-3. Historical control data (a) for tumour incidences in liver of mice (NTP 1986; Krishna-Murthy et al., 1990) *

Laboratory	**Adenoma**	**Carcinoma**	**Adenoma or Carcinoma**
Batelle	4/98 (4.1%)	3/98 (3.1%)	6/98 (6.1%)
Gulf South	16/334 (4.8%)	11/334 (3.3%)	27/334 (8.1%)
Litton	4/148 (2.7%)	3/148 (2.0%)	7/148 (4.7%)
Mason	10/198 (5.1%)	7/198 (3.5%)	17/198 (8.6%)
Papanicolaou	2/48 (4.2%)	2/48 (4.2%)	4/48 (8.3%)
Southern	11/300 (3.7%)	7/300 (2.3%)	18/300 (6.0%)
Total	47/1126 (4.2%)	33/1126 (2.9%)	79/1126 (7.0%)
SD (b)	2.45%	2.22%	3.28%
Overal historical range			
High	5/50 (10%)	4/50 (8%)	7/50 (14%)
Low	0/50 (0%)	0/50 (0%)	1/50 (2%)

(a) data as of March 16, 1983 for studies of at least 104 weeks; the exact time period of the individual historical control data has not been specified by NTP (1986).
(b) standard deviation. Range and SD are presented for groups of 35 or more animals
* The two year mice study of NTP (1986)/Krishna-Murthy et al. (1990) were begun in March 1979 and completed in March 1981 and were conducted at EG&G Mason Research Institute.

Three other studies were identified, and presented below, in sections 3.9.1.3/3.9.1.4/3.9.1.5. However, information on the design and results of these studies was very limited and, therefore, these studies are not adequate for carcinogencity assessment. These studies are however shortly described for completeness.

3.9.1.3 Study 3: McCammon et al 1957, rat/mouse)

In an abstract McCammon et al. (1957) stated that resorcinol diglycidyl ether was carcinogenic in C57/B1 mice treated by intrascapular painting three times a week and in Long-Evans rats exposed to the substance by subcutaneous injection.

3.9.1.4 Study 4: Kotin and Falk (1963) -mouse

In a study by Kotin and Falk (1963), 20 C57/B1 mice received a concentration of 0.75 mM resorcinol diglycidyl ether (administration route and duration not reported). One skin tumour was observed after 8 months when 14 mice were still alive. No additional tumours (malignant lymphoma or pulmonary adenoma) were observed in the exposed animals.

3.9.1.5 Study 5: Van Duuren et al. (1965) - mouse

Van Duuren et al. (1965) exposed 30 female Swiss-Millerton mice to 1% resorcinol diglycidyl ether in benzene by dermal application three times per week for their entire life-span (median survival time: 491 days). No skin tumours (papilloma or squamous epidermoid carcinoma) were observed.

3.9.2 Human data

No data on carcinogenicity in humans were found.

3.9.3 *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

3.9.3.1 Cell transformation study (Matthews et al., 1993a/b)

In both trials of a transformation assay using the A31-1-13 clone of BALB/c-3T3 cells, a statistically significant transformation response was observed (Matthews et al., 1993a/b).

Table 3.9.3.1-1 Cell transformation studies with resorcinol diglycidyl ether

Method	Cell type	Concentration	Results and remarks	Reference
Transformation	A31-1-13 clone of BALB/c- 3T3 cells	*Method*: Tests performed in duplicate *Concentrations*: 0, 2.18, 4.08, 6.26, 9.53 (first trial) 0, 1.36, 2.72, 4.08, 5.44 µM (second trial) *Metabolic activation*: Not used *Controls*: Negative: vehicle; Positive: benzo(a)pyrene *Purity*: unknown *Solvent*: Dissolved in DMSO at a high concentrations and then dispersed in medium supplemented with a noncytotoxic, nonionic surfactant	*Outcome*: positive Transformation response (Foci/Vessel; focus type III): 0.348, 3.49 ($p \leq 0.001$), 11.8 ($p \leq 0.001$), 1.51, 0 (1st trial) 0.16, 0.392 ($0.01 < p \leq 0.05$), 0.842 ($0.01 < p \leq 0.05$), 3.45 ($p \leq 0.001$), 3.45 ($p \leq 0.001$) (2nd trial) *Cytotoxicity*: Relative cloning efficiency: 100, 80, 19.4, 0.588, 0% (1st trial), 100, 104, 70.4,	Matthews et al., 1993a/b Klimisch score 2

		pluronic F68 (final concentrations max. 0.2% v/v DMSO and 0.25% w/w F68) *Statistical analysis*: (1) ANOVA on log10 transformed data using the F-test, followed by modified Student's t-test (model for unequal or equal variances); Individual treatments were compared with vehicle control by the appropriate unequal variance or equal variance t-statistic	18.9, 2.2% (2nd trial), for control and lowest through highest concentration, respectively	

3.9.4 Other data (e.g. studies on mechanism of action)

N/A

3.10 Reproductive toxicity

This hazard class has not been evaluated.

3.11 Specific target organ toxicity – single exposure

This hazard class has not been evaluated.

3.12 Specific target organ toxicity – repeated exposure

This hazard class has not been evaluated. However, in support of the evaluation of the endpoint carcinogencity, the individual studies can be found below.

3.12.1 Animal data

3.12.1.1 Study 1 (oral, rat. 2-week): Ghanayem et al., (1986)

Male Fischer 344 rats (8-16/group) were administered resorcinol diglycidyl ether via oral gavage at dose levels of 0, 12 and 25 mg/kg bw/d for 5 days/week, 2 weeks (Ghanayem et al., 1986). Control rats were given corn oil via the same protocol. The dose levels applied in this study corresponded to the ones applied in the 2-year carcinogenicity studies of NTP (see section 3.9). Twenty-four hours after the last dose, rats were anesthetized with ether, an abdominal incision was made, and the entire stomach was removed and processed. The stomach was fixed in 10% aqueous neutral buffered formalin. After fixation, the stomach was trimmed into 5-7 sections, processed using routine procedure, and embedded in paraffin. Histologic sections were stained with hematoxylin and eosin, coded, and evaluated by a pathologist without prior knowledge of the treatment.

Histopathological examination of the forestomachs revealed that resorcinol diglycidyl ether caused a significant increase in the incidence and severity of mucosal cell proliferation and hyperkeratosis at the high dose of 25 mg/kg bw/d (Table 3.12.1.1-1). Epithelial cell proliferation and hyperkeratosis of the forestomach as induced by resorcinol diglycidyl ether was multifocal in nature. In general, the proliferative changes observed in the mucosa were more pronounced toward the proximal (esophageal) end of the forestomach with gradual diminution in severity in more distal aspects of the forestomach.

Table 3.12.1.1-1. Incidence of forestomach epithelial cell proliferation and hyperkeratosis induced by gavage administration of resorcinol diglycidyl ether (Ghanayem et al., 1986)

Dose (mg/kg bw/d)	**Cell proliferation**		**Hyperkeratosis**	
	Incidence	***P****	**Incidence**	***P****
12	0/8	-	0/8	-
25	4/8	0.024	5/8	0.01

* Incidence were compared with control using one-sided (*P*) Fischer Exact Test

3.12.1.2 Study 2 (oral, rat, 2-week): NTP (1986); Krishna-Murthy et al., 1990

Male and female F344/N rats (5/sex/dose) were administered resorcinol diglycidyl ether via oral gavage at daily doses of 0, 190, 380, 750, 1500 or 3000 mg/kg bw/d for 14 consecutive days (NTP, 1986; Krishna-Murthy et al., 1990). Animals were observed twice daily for mortality. Necropsies were performed on all animals.

All males and females that received 750, 1500 or 3000 mg/kg bw/d and 2/5 males that received 380 mg/kg bw/d died before the end of the study (table 3.12.1.2-1). All rats receiving 380 mg/kg bw/d and 2/5 males and 1/5 females receiving 190 mg/kg bw/d lost weight during the study. Clinical signs were considered by the study authors as not compound related. Macroscopically observable effects were found in the kidney and stomachs of rats administered resorcinol diglycidyl ether (Table 3.12.1.2-2). The reneal medullae were red and more prominent than usual. The forestomach showed reddened mucosae and early development of small papillary-like growths. No histopathologic examinations were performed to further characterize these lesions.

Tabel 3.12.1.2-1. Survival and mean body weight of rats administered resorcinol diglycidyl ether in corn oil by gavage for 14 days (NTP 1986)

Dose (mg/kg bw/d)		**Mean body weight (g)**			**Final body weight relative to controls([c]) (%)**
	Survival ([a])	**Initial**	**Final**	**Change ([b])**	
Males					
0	5/5	156.4 ± 4.3	176.2 ± 7.1	+19.8 ± 3.0	-
190	5/5	156.2 ± 4.5	156.6 ± 4.5	+0.4 ± 1.4	-11
380	3/5	151.7 ± 2.6	136.0 ± 1.7	-15.7 ± 2.4	-23
750	0/5	([d])	([d])	([d])	([d])
1500	0/5	([d])	([d])	([d])	([d])
3000	0/5	([d])	([d])	([d])	([d])
Females					
0	5/5	118.0 ± 5.5	130.8 ± 7.3	+12.8 ± 1.9	-
190	5/5	117.4 ± 5.6	119.4 ± 6.2	+2.0 ± 0.8	-9
380	5/5	117.8 ± 3.9	109.8 ± 5.4	-8.0 ± 2.0	-16
750	0/5	([d])	([d])	([d])	([d])
1500	0/5	([d])	([d])	([d])	([d])
3000	0/5	([d])	([d])	([d])	([d])

(a) Number surviving/number per group. All calculations are based on those animals surviving to the end of the study.
(b) Mean weight change of the survivors of the group ± standard error of the mean
(c) Final body weight relative to controls =

$$\frac{\text{Final weight (dosed group)} - \text{Final weight (control group)}}{\text{Final weight (control group)}} \times 100\%$$

(d) No data are presented due to the 100% mortality in this group.

Table 3.12.1.2-2. Incidences of some compound-related effects in rats administered resorcinol diglycidyl ether in corn oil by gavage for 14 days (NTP 1986).

Dose (mg/kg bw/d)	**Observation**		
	Renal medullae – dark red	**Glandular stomach – reddened mucosa**	**Forestomach – papillary growth**
Males			
0	0/5	0/5	0/5
190	0/5	0/5	0/5
380	2/5	2/5	1/5
750	3/5	5/5	0/5
1500	5/5	5/5	0/5
3000	0/5	5/5	0/5
Females			
0	0/5	0/5	0/5
190	0/5	0/5	5/5
380	0/5	0/5	4/5
750	5/5	5/5	0/5
1500	5/5	4/5	0/5
3000	5/5	2/5	0/5

3.12.1.3 Study 3 (oral, rat, 13-week): NTP (1986); Krishna-Murthy et al., 1990

Male and female F344/N rats (10/sex/dose) were administered resorcinol diglycidyl ether via oral gavage doses of 0, 12.5, 25, 50, 100 or 200 mg/kg bw/d, 5 days per week for 13 weeks (NTP, 1986; Krishna-Murthy et al., 1990). Animals were checked for mortality and signs of morbidity twice daily. Those animals that were judged to be moribund were killed with carbon dioxide and necropsies were performed. Each animal were given a weekly clinical examination, including a palpation for tissue masses or swelling. Body weight data were collected weekly. At the end of the 13-week study, survivors were killed and necropsies were performed on these animals and on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. The following specimens were examined for the control and the two highest dose groups: gross lesions, tissue masses, abnormal lymph nodes, skin, mandibular lymph nodes, mammary gland, salivary gland, sternebrae, bone marrow, thymus, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, small intestine, colon, mesenteric lymph nodes, liver, pancreas, spleen, kidneys, adrenals, uri- nary bladder, prostate/ testes or ovaries/ uterus, brain, pituitary, and spinal cord. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Because of chemical-related lesions found in the higher dose groups, histologic examinations were also performed on the stomachs of male and female rats that received 12.5, 25, or 50 mg/kg bw/d.

Compound-related lesions were observed in the forestomach (squamous cell papilloma, hyperkeratosis, and basal cell hyperplasia) and the liver (minimal to mild centrilobular fatty metamorphosis). Chronic inflammation in the mesenteric lymph nodes was probably seconday to the inflammation or ulceration of the forestomach. Compared with the controls, the three male rats with fatty metamorphosis in the liver had decreased final body weights. However, lower mean body weight gains were also found in other male and female rats administered 200 mg/kg bw/d which did not show hepatic fatty metamorphosis.

At necropsy, the wall of the forestomach was sometimes thickened and the mucosal surface contained small, white papillomatous nodules. When examined microscopically, some nodules and squamous papillomata, having localized acanthosis and papillary projections of the epidermis covered by thick layers of keratinized cells. The basal layer of the epithelium was hyperplastic, sometimes showing finger-like projections into the submucosa. Diffuse hyperkeratosis, focal basal cell hyperplasia, or both were usually present in the forestomach of rats without discrete squamous papillomata. In some rats that received 200 mg/kg bw/d, ulceration in the forestomach had completely eroded the epithelium and extended into the muscularis. A few rats without ulcers had circumscribed areas of inflammation in the stomach.

Table 3.12.1.3-1. Survival and mean body weight of rats administered resorcinol diglycicyl ether in corn oil by gavage for 13 weeks (NTP, 1986)

Dose (mg/kg bw/d)		**Mean body weight (g)**			**Final body weight relative to controls([c]) (%)**
	Survival ([a])	**intial**	**final**	**Change ([b])**	
Males					
0	10/10	127.4 ± 2.6	323.0 ± 7.6	+195.6 ± 6.4	
12.5	10/10	128.3 ± 2.7	324.3 ± 5.2	+196.0 ± 5.2	0
25	10/10	128.6 ± 2.5	331.1 ± 3.6	+202.5 ± 3.5	+3
50	10/10	128.1 ± 2.7	316.6 ± 5.5	+188.5 ± 4.4	-2
100	10/10	129.2 ± 2.7	292.1 ± 4.4	+162.9 ± 4.5	-10
200	9/10	128.4 ± 2.9	241.8 ± 2.2	+113.4 ± 2.4	-25
Females					
0	10/10	104.9 ± 2.0	185.7 ± 2.9	+80.8 ± 3.6	
12.5	10/10	104.9 ± 1.8	184.7 ± 2.3	+79.8 ± 2.9	-1
25	10/10	104.5 ± 1.8	181.2 ± 2.7	+76.7 ± 2.3	-2
50	10/10	105.2 ± 1.9	184.2 ± 3.4	+79.0 ± 4.1	-1
100	10/10	105.3 ± 2.0	179.5 ± 2.8	+74.2 ± 3.2	-3
200	10/10	105.0 ± 1.6	167.5 ± 3.6	+62.5 ± 2.8	-10

(a) Number surviving/number per group. All calculations are based on those animals surviving to the end of the study.
(b) Mean weight change of the survivors of the group ± standard error of the mean
(c) Final body weight relative to controls =

$$\frac{\text{Final weight (dosed group)} - \text{Final weight (control group)}}{\text{Final weight (control group)}} \times 100\%$$

Table 3.12.1.3-2 Lesions observed in rats administered resorcinol diglycicyl ether in corn oil by gavage for 13 weeks (NTP, 1986)

	Dose (mg/kg bw/d)											
	Males ([a])						**Females ([a])**					
	0	**12.5**	**25**	**50**	**100**	**200**	**0**	**12.5**	**25**	**50**	**100**	**200**
Stomach												
Inflammation	0	8	6	3	0	3	0	8	9	3	1	2
Ulcer	0	0	0	0	0	4	0	0	0	1 ([b])	0	1
Fibrosis	0	2	2	0	0	0	0	0	0	0	0	0
Hyperkeratosis	0	1	1	7	9	7	0	0	0	1	9	7
Basal cell hyperplasia	2	3	5	7	9	7	1	3	2	5	7	7
Squamous papilloma	0	0	0	1	1	3	0	0	0	0	1	2
No lesion seen	8	1	1	0	0	0	9	1	0	3	0	0
Lymph node												
inflammation	0	NE ([c])	NE	NE	NE	7	0	NE	NE	NE	NE	4
Liver												
Fatty metamorphosis, mild or slight	0	NE	NE	NE	NE	3	0	NE	NE	NE	NE	0

(a) Ten animals were examined in each dose group
(b) Ulcer was shallow lesion of glandular stomach, not forestomach
(c) NE = not examined

3.12.1.4 Study 4 (oral, mouse, 2-week): NTP (1986); Krishna-Murthy et al., 1990

Male and female B6C3F1 mice (5/sex/dose) were administered resorcinol diglycidyl ether via oral gavage daily doses of 0, 90, 190, 380, 750 or 1500 mg/kg bw/d for 14 consecutive days (NTP, 1986; Krishna-Murthy et al., 1990). Animals were observed twice daily for mortality. Necropsies were performed on all animals.

Five of five males and 4/5 females receiving 1500 mg/kg bw/d and 2/5 males receiving 750 mg/kg bw/d died (Table 3.12.1.4-1). These deaths were attributed to administration of resorcinol diglycidyl ether. Wieght loss was observed in all mice that received 750 mg/kg bw/d or more and in 4/5 males en 1/5 females thet received 380 mg/kg bw/d. Weight loss occurred in mice in the 90 mg/kg bw/d groups (4 males and 5 females), but not in animals administered 190 mg/kg bw/d. Clinical signs were not considered to be compound related according to the study authors. Compound-related effects were observed grossly in the kidney (reddened medullae) and stomach (reddened mucosae) (Table 3.12.1.4-2). No histopathological examinations were performed to further characterize these lesions.

Table 3.12.1.4-1. Survival and mean body weight of mice administered resorcinol diglycidyl ether in corn oil by gavage for 14 days (NTP (1986).

Dose (mg/kg bw/d)		**Mean body weight (g)**			**Final body weight relative to controls[c] (%)**
	Survival [a]	**intial**	**final**	**Change [b]**	
Males					
0	5/5	23.6 ± 0.4	24.8 ± 0.8	+1.2 ± 0.4	-
90	4/5	23.6 ± 0.9	21.5 ± 1.4	-2.1 ± 1.3	-13
190	5/5	23.3 ± 0.7	25.9 ± 0.9	+2.6 ± 0.5	+4
380	5/5	23.6 ± 0.6	22.4 ± 0.8	-1.2 ± 0.6	-10
750	3/5	23.2 ± 1.1	18.3 ± 2.0	-4.9 ± 1.3	-26
1500	0/5	([d])	([d])	([d])	
Females					
0	5/5	19.6 ± 0.4	21.4 ± 0.8	+1.8 ± 0.5	-
90	5/5	19.5 ± 0.5	18.8 ± 0.4	-0.7 ± 0.1	-12
190	5/5	19.4 ± 0.6	20.7 ± 0.6	+1.3 ± 0.2	-3
380	5/5	19.3 ± 0.4	19.9 ± 0.6	+0.6 ± 0.3	-7
750	5/5	19.4 ± 0.3	18.0 ± 0.8	-1.4 ± 0.6	-16
1500	1/5	19.3 ± 0.0	17.2 ± 0.0	-2.1 ± 0.0	-20

(a) Number surviving/number per group. All calculations are based on those animals surviving to the end of the study.
(b) Mean weight change of the survivors of the group ± standard error of the mean
(c) Final body weight relative to controls =

$$\frac{\text{Final weight (dosed group)} - \text{Final weight (control group)}}{\text{Final weight (control group)}} \times 100\%$$

(d) No data are presented due to the 100% mortality in this group.

Table 3.12.1.4-2. Incidences of compound-related effects in mice administered resorcinol diglycidyl ether in corn oil by gavge for 14 days (NTP (1986).

Dose (mg/kg bw/d)	**Observation**		
	Renal medullae – dark red	**Glandular stomach – reddened mucosa**	**Forestomach – pappilary growth**
Males			
0	0/5	0/5	0/5
90	1/5	0/5	0/5
190	1/5	0/5	0/5

Dose (mg/kg bw/d)	Observation		
	Renal medullae – dark red	Glandular stomach – reddened mucosa	Forestomach – pappilary growth
380	0/5	0/5	4/5
750	2/5	2/5	1/5
1500	5/5	5/5	0/5
Females			
0	0/5	0/5	0/5
90	0/5	0/5	0/5
190	0/5	0/5	0/5
380	2/5	0/5	2/5
750	3/5	1/5	3/5
1500	0/5	4/5	0/5

3.12.1.5 Study 5 (oral, mouse, 13-week): NTP (1986); Krishna-Murthy et al., 1990

Male and female B6C3F1 mice (10/sex/dose) were administered resorcinol diglycidyl ether via oral gavage doses of 0, 25, 50, 100, 200 or 400 mg/kg bw/d, 5 days per week for 13 weeks (NTP, 1986; Krishna-Murthy et al., 1990). Animals were checked for mortality and signs of morbidity twice daily. Those animals that were judged to be moribund were killed with carbon dioxide and necropsies were performed. Each animal were given a weekly clinical examination, including a palpation for tissue masses or swelling. Body weight data were collected weekly. At the end of the 13-week study, survivors were killed and necropsies were performed on these animals and on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. The following specimens were examined for the control and highest dose group: gross lesions, tissue masses, abnormal lymph nodes, skin, mandibular lymph nodes, mammary gland, salivary gland, sternebrae, bone marrow, thymus, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, small intestine, colon, mesenteric lymph nodes, liver, gallbladder, pancreas, spleen, kidneys, adrenals, uri- nary bladder, prostate/ testes or ovaries/ uterus, brain, pituitary, and spinal cord. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Because of chemical-related lesions found in the higher dose groups, histologic examinations were also performed on the stomachs of male and female mice that received 50, 100, or 200 mg/ kg bw/d. The liver, kidneys, and testes of mice that received 200 mg/ kg were examined histologically.

Nine of ten males and 7/10 females receiving 400 mg/kg bw/d died; these deaths were considered to be compound related. Final mean body weight compared to controls was depressed 10-25% in groups that received 400 mg/kg bw/d (Table 3.12.1.5-1). Clinical signs were considered not to be compound-related. Compound-related lesions were found in the forestomach and liver of male and female mice (Table 3.12.1.5-2).

The effects seen in the forestomach resembled those seen in rats (section 3.12.1.3); squamous papillomata, diffuse hyperkeratosis, basal cell hyperplasia, and inflammation. Two females administered 400 mg/kg bw/d had mucosal ulcers of the forestomach.

Slight to mild focal tubular atrophy of the testes was seen in three mice that died during weeks 9 or 10. This lesion was not seen in mice that survived to the end of the study. The mean body weight of the male mice receiving 400 mg/kg bw/d was 26.0 g at week 8 (10 mice alive) and 27.4 g at week 9 (5 mice alive), whereas the mean body weights of all other groups of males mice for these same time periods ranged from 31.1 to 32.4 g. For these reasons, the testicaly atrophy was interpreted as being the result of morbidity rather than a direct effect or resorcinol diglycidyl ether.

Liver lesions were observed in the high dose mice only. Hepatic necrosis was focally extensive, involving large areas of the liver that were sharply demarcated from the nonnecrotic liver. Smaller, multiple areas of necrosis were seen in some mice. Minimal to mild fatty metamorphosis was observed in periportal areas of the liver, but only in animals that died.

Table 3.12.1.5-1. Survival and mean body weight of mice administered resorcinol diglycicyl ether in corn oil by gavage for 13 weeks (NTP, 1986)

Dose (mg/kg bw/d)	**Survival ([a])**	**Mean body weight (g)**			**Final body weight relative to controls([c]) (%)**
		intial	**final**	**Change ([b])**	
Males					
0	10/10	22.1 ± 0.3	35.1 ± 0.8	+13.0 ± 0.7	
25	10/10	22.1 ± 0.4	35.6 ± 0.6	+13.5 ± 0.3	+1
50	10/10	21.6 ± 0.4	35.3 ± 0.8	+13.7 ± 0.5	+1
100	10/10	21.9 ± 0.5	34.0 ± 0.8	+12.1 ± 0.5	-3
200	10/10	22.1 ± 0.3	32.7 ± 0.5	+10.6 ± 0.5	-7
400	1/10	21.1 ± 0.0	26.5 ± 0.0	+5.4 ± 0.0	-25
Females					
0	10/10	17.7 ± 0.5	24.8 ± 0.6	+7.1 ± 0.7	
25	10/10	18.1 ± 0.5	26.9 ± 0.9	+8.8 ± 0.7	+8
50	10/10	18.2 ± 0.3	25.4 ± 0.4	+7.2 ± 0.3	+2
100	10/10	18.4 ± 0.4	25.7 ± 0.7	+7.3 ± 0.5	+4
200	10/10	18.0 ± 0.2	25.2 ± 0.6	+7.2 ± 0.5	+2
400	3/10	19.1 ± 0.9	22.4 ± 0.9	+3.3 ± 0.5	-10

(a) Number surviving/number per group. All calculations are based on those animals surviving to the end of the study.
(b) Mean weight change of the survivors of the group ± standard error of the mean
(c) Final body weight relative to controls =

$$\frac{\text{Final weight (dosed group)} - \text{Final weight (control group)}}{\text{Final weight (control group)}} \times 100\%$$

Table 3.12.1.5-2 Lesions observed in mice administered resorcinol diglycicyl ether in corn oil by gavage for 13 weeks (NTP, 1986)

	Dose (mg/kg bw/d)											
	Males ([a])						**Females ([a])**					
	0	**25**	**50**	**100**	**200**	**400**	**0**	**25**	**50**	**100**	**200**	**400**
Stomach												
Inflammation	0	0	1	0	3	0	0	4	2	0	4	2
Ulcer	0	0	0	0	0	0	0	0	0	0	0	2
Basal cell hyperplasia	0	1	0	2	1	2	0	0	0	1	6	2
Hyperkeratosis	0	0	1	7	4	8	0	0	3	7	8	5
Squamous hyperplasia	0	1	0	0	0	0	0	0	0	0	0	0
Squamous papilloma	0	0	1	0	5	2	0	0	0	1	1	5
Epidermal inclusion cyst	0	2	0	0	0	0	0	0	0	0	0	0
No lesion seen	10	8	8	3	0	0	10	6	6	2	0	0
Liver												
Focal necrosis	0	NE([b])	NE	NE	0	5	0	NE	NE	NE	0	3
Fatty metamorphosis	0	NE	NE	NE	0	3	0	NE	NE	NE	0	1
Focal inflammation	1	NE	NE	NE	0	0	0	NE	NE	NE	0	0
Testis												
Focal tubular atrophy	0	NE	NE	NE	0	3						

(a) Ten animals were examined in each dose group
(b) NE = not examined

3.12.2 Human data

N/A

3.12.3 Other data

N/A

3.13 Aspiration hazard

This hazard class has not been evaluated.

4 ENVIRONMENTAL HAZARDS

This hazard class has not been evaluated.