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:

Reaction mass of 1,3-dioxan-5-ol and

1,3-dioxolan-4-ylmethanol (glycerol formal)

EC Number: **N/A**

CAS Number: **N/A**

Index Number: **603-RST-VW-Y**

Contact details for dossier submitter:

Bureau REACH
National Institute for Public Health and the Environment (RIVM)
The Netherlands
bureau-reach@rivm.nl

Version number: 2.0 **Date: September 2021**

CONTENTS

1 PHYSICAL HAZARDS

Evaluation not performed for this substance.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Evaluation not performed for this substance.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

Evaluation not performed for this substance.

3.2 Acute toxicity - dermal route

Evaluation not performed for this substance.

3.3 Acute toxicity - inhalation route

Evaluation not performed for this substance.

3.4 Skin corrosion/irritation

Evaluation not performed for this substance.

3.5 Serious eye damage/eye irritation

Evaluation not performed for this substance.

3.6 Respiratory sensitisation

Evaluation not performed for this substance.

3.7 Skin sensitisation

Evaluation not performed for this substance.

3.8 Germ cell mutagenicity

Evaluation not performed for this substance.

3.9 Carcinogenicity

Evaluation not performed for this substance.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Study 1

Study reference:

ST 2060 - 3-months toxicity test with oral administration in rats, study report from 1973, reported as study 001 in IUCLID´s section on repeated oral toxicity

Detailed study summary and results:

Test type

Similar to OECD TG 408, deviations from the guideline: ophthalmological examinations, functional observation and water consumption were not recorded

No statement on GLP is given.

Test substance

- Glycerol formal (ST 2060)
- Impurities: stabilised with 0.02% ethylenediamine tetraacetic acid-sodium salt; 0.02% propylgallate and 0.01% thiodipropionic acid
- Mixture in constant ration of 5-hydroxy-1,3-dioxane (60%) and 4-hydroxymethyl-1,3-dioxolane (40%)

Test animals

- Male and female Sprague Dawley rats
- 10 animals/sex /dose
- 2 additional animals/sex /dose were treated comparably but held for six weeks after end of exposure to examine regeneration effects.
- Source: own SPF-breeding
- Age at study initiation: not specified
- Weight at study initiation: 182-202 g for females; 252-295 for males
- Fasting period before study: not specified

- Housing: Separation of animals according to sex. Animals were kept in pairs in Macrolon containers of type III in a fully air-conditioned test room.
- Diet: ALTROMIN-R-powder ad libitum
- Water: ad libitum
- Acclimation period: not specified

- ENVIRONMENTAL CONDITIONS
- Temperature (°C): 24 +/-1
- Humidity (%): 50-60
- Air changes (per hr): 15-20
- Photoperiod (hrs dark / hrs light): 10/10

Administration/exposure

- Oral via gavage
- Daily for 90 days
- 0, 0.01; 0.10 and 1.00 mL/kg bw/d (Using the density of 1.218 g/mL, the following doses were calculated by the dossier submitters: 0, 12, 121, 1218 mg/kg bw/d)
- Control animals treated with water
- No historical control data provided
- Vehicle: water (dilutions were made in a way that always 2 mL/kg bw/d could be applied for all doses)

Description of test design:

- No mating performed
- Observations and tests:
    - Daily observation of general behaviour
    - Daily control of weight
    - Weekly control of consumed quantity of feed
    - Haematological tests
    - Medico-chemical tests
        - Enzyme activities (e.g. alkaline phosphatase in plasma and liver)
        - Lipid content of plasma and liver
        - Total protein and albumin fractions in the plasma
        - Other components of the blood
        - Sulfhydryl groups in the liver
    - Urinalysis

- Liver function test with phenacetin
- Renal function test with phenol red
- Determination of the leucin amino peptidase activity in the urine
- Histopathological studies including reproductive organs

Results and discussion

Only data relevant for the endpoint reproductive toxicity is described in this section.

- 4 animals died in the high dose group (2/12 males and 2/12 females). No other clinical signs were observed.
- At the end of exposure, the gain in weight in percent was 89.74, 94.87 and 66.67% (females) and 80.87, 68.31 and 57.36% (males) (low, middle, high dose group)
- Absolute feed efficiency as well as the relative feed efficiency in % of control showed a decrease depending on dose. For details see the following table.

Table 1: Mortality, body weight and feed consumption data

	sex	0 mg/kg bw/d	12 mg/kg bw/d	121 mg/kg bw/d	1218 mg/kg bw/d
Mortality	♀	0/12	0/12	0/12	2/12
Mean initial weight in g	♀	190	182	183	202
Mean weight in g after 3 months	♀	268	252	257	254
Mean increase of single weight in g	♀	78	70	74	52
Relative weight gain in %of control	♀	-	89.74	94.87	66.67
Mean feed consumption (g/animal)	♀	1310	1250	1272	1181
Mean feed-efficiency (feed-consumption/weight gain)	♀	5.95	5.60	5.82	4.40
Relative feed efficiency in % of control	♀	100	94.11	97.82	73.95
mortality	♂	0/12	0/12	0/12	2/12
Mean initial weight in g	♂	252	274	294	295
Mean weight in g after 3 months	♂	435	422	419	400
Mean increase of single weight in g	♂	183	148	125	105
Relative weight gain in %of control	♂	-	80.87	68.31	57.38
Mean feed consumption (g/animal)	♂	1934	1927	1846	1684
Mean feed-efficiency (feed-consumption/weight gain)	♂	9.46	7.68	6.77	6.24
Relative feed efficiency in % of control	♂	100	81.18	71.56	65.96

- Dose-dependent decreases in relative organ weights (in percent of body weight) reported for uterus, seminal vesicles, testes and epididymis

Table 2: Relative Organ weights (reported in % of body weight)at the end of 90 day exposure duration

Organ	sex	0 mg/kg bw/d	12 mg/kg bw/d	121 mg/kg bw/d	1218 mg/kg bw/d
uterus	♀	0.19	0.16	0.16	0.14
ovaries	♀	0.040	0.035	0.043	0.038
thyroid	♀	0.008	0.009	0.008	0.009
seminal vesicles	♂	0.15	0.12	0.12	0.095
testes	♂	0.87	0.89	0.89	0.52
epididymis	♂	0.30	0.29	0.27	0.18
thyroid	♂	0.006	0.006	0.005	0.006

- Histopathological examination:
  - Control animals (also included the recovery animals):

    Ovary: (animals no. 2343 – 2352 and 2441

    The female gonads contain apart from numerous primary follicles in the cortex all stages of growth up to bigger, in 2344 and 2347 also especially big (ready to burst) tertiary follicles. The content of interstitial cells lies within the variation of cycling ovaries. In each organ there are several generations of corpora lutea.

    Uteri: of all animals show a normal parietal structure with partly stretched, partly slightly winded glands in the endometrium.

    Testes: (animals 2553 – 2362, as well as 2442 and 2343)

    In the tubuli contorti seminiferi of all testes all stages of normal spermiogenesis taking an undulatory course and interstitial cells according to the norm are found.

    Epididymes: Show without exception the usual structure and a rich accumulation of sperm in the ductus epididymis.

    Seminal vesicles: show a richly folded mucosa with highly prismatic epithelial tapetum and in the lumen a greatly oxyphilic cell-free secretion

  - 12 mg/kg bw/d group

    Ovaries: (animals 2363 – 2372)

    Do not differ from the control organs. In 2368 and 2371 follicles ready to burst and in 2366 quite fresh corpora lutea can be seen.

    Uteri: are perfect with two exceptions: The cavity of 2364 and 2368 is wide with low folds and partly more importantly enlarged glands in the endometrium. The latter is more importantly interspersed with polymophonuclear leucocytes especially in the superficial layer which also appear in the secretion and in the epithelium of the glands.

    Testes: (animals 2373 – 2382)

are perfect with one exception: 2373 shows in several tubular cross-sections a slight inhibition of spermiogenesis. The majority of the tubules yet shows all phases of spermiogenesis and spermiohistogenesis in the usual form.

Epididymes and seminal vesicles of all animals show no deviations from the norm.

- 121 mg/kg bw/d group

  Ovaries: (animals 2383 – 2392)

  There are no detectable differences in comparison to the control organs; 2385 and 2387 contain big tertiary follicles ready to burst.

  Uteri: show with the exception of 2385, 2386 and 2388 normal parietal layers. In the uteri of the three mentioned animals especially the superficial layer of the endometrium is more importantly interspersed with leucocytes, which partly are also found in the lumen and in the epithelium of the sporadically enlarged glands.

  Testes: (2393 - 2402)

  The organs show a normal undisturbed spermiogenesis with 2 exceptions. 2394 and 2399 show in several tubular cross sections an inhibited spermiogenesis and 2399 has additionally some already completely atrophic tubules with simple epithelium.

  Epididymis and seminal vesicles of all animals of this group show no detectable changes in comparison to the control organs.

- 1218 mg/kg bw/d group

  Ovaries: (animals 2403 - 2410)

  The organs show no conspicuous differences in comparison to those of the control group. 2404 and 2406 contain big tertiary follicles which yet are not ready to burst. No fresh corpora lutea were found.

  Uteri: show a normal parietal structure with exception of 2405. In 2405 the endometrium is more importantly interspersed with lobular-nuclear leucocytes. The latter appear also in the epithelium and in the lumen of the sporadically wide-luminal glands.

  Testes: (2411 - 2418)

  All organs show changes in different extent. They are the most negligible in 2413 and 2416, where the majority of the tubular cross-sections shows a normal spermiogenesis.

  In 2416 the spermiogenesis is inhibited in some tubules, 2413 shows additionally some atrophic tubules with simple epithelium. In 2415 and 2418 the above changes appear in greater number. In 2411 and 2412 there is a subtotal orchiatrophy, where only few tubules show stages of spermiogenesis and in 2414 and 2417 there occurred practically a total orchiatrophy. All organs show additionally in fluctuating extent an interstitial edema.

Epididymis: show changes parallel to the testes. Whereas the epididymal tubules of 2413 and 2416 and their content show not yet any differences in comparison to the control organs, 2415 and 2418 show with morphologically unchanged tubular epithelium an abnormal content in the ductus epididymidis consisting of degenerated cells of the seminal epithelium besides few sperma. In the other organs, especially in 2414 and 2417 the cross-section of the epididymal tubules is smaller than in the control organs and the epithelium is lower. In the ductus epididymidis there are apart from singular spermatozoa and degenerated cells from the seminal epithelium crumbly oxyphilic masses.

Seminal vesicles of 2413, 2415, 2416 and 2418 are apparently unchanged. In the organs the mucosal folds and the epithelium are lower. There is plenty of secretion in 2411, 2414 and 2417 which is mixed with swarms of desquamated cells.

- Recovery group:
  - Control animals:

    Included above

  - 12 mg/kg bw/d group

    Ovaries: (animals 2444, 2445) without finding

    Uteri: No findings in the uterus of 2445. The organ of 2444 is mainly in the superficial parts of the endometrium more importantly interspersed with lobular-nuclear granulocytes, which appear also in the epithelium and in the lumen of the sometimes dilated gland tubes.

    Testes, epididymes and seminal vesicles: (animals 2446 and 2447) of all animals show no deviations from the norm.

  - 121 mg/kg bw/d group

    Ovaries and uteri: (animals 2448 and 2449) do not differ from the control group

    Testes: (animals 2450 and 2451)

    The testes of 2451 are perfect. In 2450 the majority of the tubular cross sections shows a normal spermiogenesis, apart from this yet there are also atrophic tubules in low number. Among them a slight interstitial oedema can be seen.

    Epididymis and seminal vesicles of all animals of this group show no perceptible deviations from the controls.

  - 1218 mg/kg bw/d group

    Ovaries and Uteri: (animals 2452 and 2453) show no abnormal findings

    Testes: (animals 2454 and 2455)

In the testes of both animals apart from tubuli contorti with normal spermiogenesis also such with inhibited spermiogenesis and single completely atrophic tubules are found. Traces of an interstitial oedema are seen in both animals.

Epididymis: show with normally appearing tubules in the ductus epididymidis single degenerated cells from the seminal epithelium besides plenty of spermatozoa with morphologically normal appearance

Seminal vesicles: do not show any differences in comparison to the control organs

3.10.1.2 Study 2

Study reference:

Glycerol Formal. Oral Reproduction Study in Female Rats, study report from 1982, not reported in the IUCLID file

Detailed study summary and results:

Test type

Females were exposed 14 days prior to mating (with unexposed males), during mating and gestation until PND 20 of pups. The pups were used for a 90 day oral toxicity study.

Statement on GLP is given.

Test substance

- Glycerol formal (purity: >99%)

Test animals

- Male and female Charles River CD rats
- 2 0female animals/dose, males untreated
- Source: Charles River France, 76410 Saint Aubin les Elbeuf, France
- Age at study initiation: 16 weeks (females)
- Weight at study initiation: 214 to 247 g (females)
- Fasting period before study: not specified
- Housing: Cages in an air-controlled room.
- Diet: Certified UAR"A 04" chow ad libitum.
- Water: tap water ad libitum
- Acclimation period: not specified

- ENVIRONMENTAL CONDITIONS

- Temperature (°C): not specified
- Humidity (%): not specified
- Air changes (per hr): not specified
- Photoperiod (hrs dark / hrs light): 12/12

Administration/exposure

- Oral via gavage
- Doses: 0, 1, 5, 25 mg/kg bw/d
- 5 ml/kg bw of solution (treated females) or vehicle (control females). Doses were adjusted based on most recent body weights.
- Solutions prepared daily, stability and concentration of drug solutions confirmed by chemical analysis.
- Control animals treated with water
- After 15 days of administration, females were placed with untreated males (2 females per male) and dosing continued throughout the mating period. Upon detection of spermatozoa in the daily vaginal lavage, (day 0 of gestation), mated females were placed in individual cages and dosing continued through gestation until PND20
- No historical control data provided
- Vehicle: distilled water

Description of test design:

- Males untreated
- Females were sacrificed if they did not mate within an 8-day period. In addition, if females failed to deliver by Day 24 of gestation, they were sacrificed and their uterine contents examined.
- Observations and tests:
    - All females and all pups observed daily, with less extensive examinations on week-end and holidays.
    - Body weights of females recorded on Days -15, -8, and -1 of prebreeding period, on Days 1, 6, 14, 16, 18, 20, and 21 of gestation and on Days 2, 7, 14, and 21 during lactation.
    - Pups weighed on Days 1, 7, 14, and 21 postpartum.
    - At termination of the study, all females were sacrificed and the number of metrial glands counted.
    - On PND1 pups were counted, examined externally and sexed. Litter size was standardized by random selection where possible to 4 pups of each sex in each litter. If there were less than 4 pups of one sex, additional pups of the other sex were retained so that the litter size

was maintained at 8 offspring. Excess pups were discarded. Pups were examined daily and sexed again on Days 14, and 21.

- At the end of the study, 3 to 4-week-old pups were selected for continuation on a three-month oral toxicity study (reported as "study report 1982a" in the main report) and male fertility study (reported as "study report 1982c" in the main report and more detailed as "study 3 in this annex) . When possible, at least l·male and 1 female pup were randomly selected from each litter. The remaining pups necessary to complete the group size of 20 per sex for each treatment group were selected from litters designated by a computer-generated table.

- Statistical analyses were performed for the following parameters: female body weight changes during pre-breeding, gestation, and lactation; time to mating; verified matings/number of females; number of pregnancies per number of verified matings; postimplantation survival rate; gestation length; number of dead pups per litter; average live pup weight per litter days 1, 7, 14, 21; and average number of dead pups on day 1, days 7 to 14, 14 to 21, and 1 to 21. The computer examined the data for normality using the Wilk and Shapiro statistic and for homogeneity of variances using the Levene test. Statistical significance at P = 0.05 based on an analysis of variance after normalizing for nonparametric data when necessary. In addition, effects of the number of live pups per litter and the average length of gestation on the average live Day 1 pup weight were analyzed by single covariance analysis and the data were adjusted prior to further statistical manipulation.

Results and discussion

- Mortality: No death were observed among treated F0 females
- Physical examination: No drug-related effects were noted in dams or pups
- Body weight change: No treatment-related changes were observed
  - Females: Average body weight gain was slightly but significantly ($P \leq 0.05$) lower in females from the 25 mg/kg bw/d group compared to the control group during the Days -15 to -1 of the prebreeding period. The average body weight gain was significantly increased ($P \leq 0.05$) relative to the controls in this group on Days 6 to 14 and 1 to 20 of gestation. Since these differences in mean weight gain between control and drug treated groups were very slight, and not consistent during the prebreeding and gestation and lactation periods, they were not considered treatment-related. For details see Table 3.

Table 3: Average female body weight

Dose in mg/kg bw/d	0	1	5	25
Average body weight (g) during prebreeding				
Day -15	238	240	236	241
Day - 8	248	250	248	247

Day - 1	255	258	254	255
CHANGE				
Days -15 to -8	10	10 NS	12 NS	6 S
Days -8 to -1	7	8 NS	6 NS	8 NS
Days -15 to -1	17	18 NS	18 NS	14 S
Average body weight (gm) during gestation				
Day 1	265	272	267	269
Day 6	286	298	290	291
Day 14	324	340	331	335
Day 16	342	358	348	354
Day 18	368	386	374	384
Day 20	397	415	401	414
CHANGE				
Days 1 to 6	21	26 S	23 NS	22 NS
Days 6 to 14	38	42 NS	41 NS	44 S
Days 14 to 20	73	75 NS	70 NS	79 NS
Days 1 to 20	132	143 S	134 NS	145 S
Average body weight (gm) during lactation				
Day 2	310	327	320	317
Day 7	325	336	334	332
Day 14	347	356	351	353
Day 21	342	350	350	346
CHANGE				
Days 2 to 21	32	23 NS	30 NS	29 NS

NS = Not statistically significantly different from controls ($P > 0.05$)

S = Statistically significantly different from controls ($P \leq 0.05$)

- Pups: Average live pup weight per litter was significantly higher in pups from the 1 mg/kg bw/d group on Day 21 and in pups from the 25 mg/kg bw/d group on Days 7 and 21 post partum compared to the control group. However, there were no significant differences ($P > 0.05$) in average pup weight in the 5 mg/kg bw/d group during the lactation period. In view of the magnitude of the differences in body weight in pups from the low and high dose groups and the lack of an apparent dose-response, these changes were considered as incidental (in the study report only the individual pup data are reported, no mean available, therefore, these results are nor presented here).

- Reproductive status: No treatment-related effects on reproductive status, mating performance, length of gestation or post implantation survival rate of F0 females were observed (for details see the following Table 4).

Table 4: Reproductive status (dams)

Dose in mg/kg bw/d	0	1	5	25
Total number of females	20	20	20	20
Live pregnant	16	18	16	17
Live not pregnant	2	1	1	0
Died pregnant	0	0	0	0

Died not pregnant	0	0	0	0
Sacrificed pregnant	0	0	1	0
Sacrificed not pregnant	0	0	0	0
Aborted	0	0	0	0
Died incomplete parturition	0	0	0	0
Sacrificed incomplete parturition	0	0	1	0
Died postpartum	0	0	0	0
Sacrificed postpartum	0	0	0	0
Not bred	2	1	1	3
Sacrificed	0	0	0	0
Died	0	0	0	0
Total number of identified matings/total number of females	18/20	19/20 NS	19/20 NS	17/20 NS
Days 1 to 4 of breeding	17	18	18	14
Days 5 to 8 of breeding	1	1	1	3
Days 9 to 12 of breeding	0	0	0	0
* Pregnancies/ * verified matings	16/18	18/19 NS	18/19 NS	17/17 NS
Time to mating (mean based on rankits)	1.0556	1.0526 NS	1.0526 NS	1.1765 NS
Average length of gestation	13.3	18.2 NS	17.7 NS	16.9 NS
Post implantation survival rate (%)	93.54	91.99 NS	90.08 NS	91.15 NS

NS = Not statistically significantly different from controls ($P > 0.05$)

S = Statistically significantly different from controls ($P \leq 0.05$)

- Status of offspring: There were no treatment-related effects on the survival of offspring.
    - Incidental findings: The number of dead pups from Day 8 to Day 14 postpartum was significantly ($P \leq 0.05$) higher in rats from the 1 mg/kg bw/d group than in other groups. There was no increase in mortality in this group on Day 1 or Day 21 postpartum and no treatment-related increase in dead pups was seen in the 5 or 25 mg/kg bw/d groups throughout the lactation period. See the following Table 5 for details.

Table 5: Reproductive status (pups)

Dose in mg/kg bw/d	0	75	150	300
Number of litters	16	18	16	17
Day 1 postpartum				
Total No. of pups	216	239	203	243
Sex males/females (unknown)	85/131	124/115	108/95	123/118 (2)
No. Live	214	237	202	239
No. Live after reduction	128	144	123	136
No. Dead	2	2	1	4
No. Dead pups per litter	0.125	0.111	0.062	0.235 NS
No. Live per litter	13.4	13.2	12.6	14.0
Ave. Live pup weight (gm)/litter (a)	6.07	6.40 NS	6.28 NS	6.43 NS
Day 7 postpartum				
No. Live	123	136	121	135
No. Dead pups day 2 to 7 (days 1 to 7)	5 (7)	8 (10)	2 (3)	1 (5)
Ave. Live pup weight (gm)/litter (a)	11.90	12.70 NS	12.71 NS	13.06 S
Day 14 postpartum				

No. Live	123	133	121	135
No. Dead pups day 8 to 14 (days 1 to 14)	0 (7)	3 S(13)	0 (3)	0 (5)
Ave. Live pup weight (gm)/litter (a)	25.25	27.15 NS	26.14 NS	26.98 NS
Day 21 postpartum				
No. Live	123	133	121	134
No. Dead pups day 15 to 21 (days 1 to 21)	0 (7)	0 (13) NS	0 (3)	1 NS(6)
Ave. Live pup weight (gm)/litter (a)	40.05	43.35 S	41.79 NS	43.47 S

(a) = Statistical significance at P = 0.05 after Adjustment for length of gestation

NS = Not statistically significantly different from controls (P > 0.05)

S = Statistically significantly different from controls (P ≤ 0.05)

3.10.1.3 Study 3

Study reference:

Fertility study Complementary to Study TT 82-601-0, study report from 1982, not reported in the IUCLID file

Detailed study summary and results:

Test type

Males used for this study were in utero exposed and subsequently exposed for 90 days to 0, 1, 5, 25 mg glycerol formal/kg bw/d. In exposure week 11 they were cohabited with untreated females (two females/male). On GD 14 females were sacrificed and reproductive parameters were recorded.
GLP statement is given.

Test substance

- Glycerol formal
- Purity: presumably >99%

Test animals

- Male and female CRCD rats (Charles River)
- Male rats: 20 animals/dose from an ongoing 14-week toxicity study were cohabited with sexually mature untreated females in drug week 11. The males were exposed throughout the mating period until drug week 14
- Female rats: 40 animals/dose group, animals were 11 weeks old and weighed between 178 and 205 g at the beginning of the study.
- Source of female rats: Charles River, France
- Fasting period before study: not specified
- Housing: Two females and one male in a plastic box during mating. Females and males were housed individually in suspended cages after confirmed mating.

- Diet: certified UAR A 04 Chow, ad libitum
- Water: tap water, ad libitum
- Acclimation period: not specified

- ENVIRONMENTAL CONDITIONS
  No information provided

Administration/exposure

- Oral via gavage
- Male animals from an ongoing 14-week toxicity study were cohabited with sexually mature untreated females in drug week 11. The males were exposed throughout the mating period until drug week 14. Females were not exposed
- Doses: 0, 1, 5, 25 mg/kg bw/d
- Control animals treated with water

Description of test design:

- Impregnation procedure: cohoused with non-exposed females. Females were checked daily by lavage for evidence of mating and the day that sperm was observed was designated Day 0 of gestation. Those females which did not show evidence of mating after 12 nights of cohabitation were removed from the male cages.
- Sacrifice of the females on GD14
- Observations and tests:
  - Daily observation of general behaviour
  - Reproductive status of dams:
    - Pregnant/not pregnant
    - Number of resorptions
    - Number of live/dead foetuses
    - Number of corpora lutea

- Statistics: parameters examined included time to mating, number of mated females/male, number of pregnant females/total number of mated females (per male), percent preimplantation loss, number of implants/pregnant female, number of resorptions/number of implants, and number of live foetuses per pregnant female. The data were analysed statistically based on an analysis of variance after an adjustment for non-parametric data, when necessary, at a significance level of P = 0.05

Results and discussion

- Physical examination: no treatment-related clinical signs were observed
- Mating performance: No substance-related effects were observed on male fertility as indicated by the time to mating, the number of mated females/total number of females and the number of pregnant females/total number of mated females (see following table).
- No effects were observed on the percent of preimplantation loss, the number of resorptions/number of implants and the number of live foetuses per pregnant female (see following table).
- A slight increase in preimplantation losses was observed in females mated to high dose males (not statistically significant). This was not considered treatment-related by the study authors but the result of a single female which had only one foetus. The second female mated with the same male had a normal litter size.

Table 6: Fertility summary table

Dose in mg/kg bw/d	0	1	5	25
Number of males	20	20	20	20
Total Number of identified matings	37	39	37	37
Days 1 to 4 of breading	34	35	36	32
Days 5 to 8 of breading	3	3	1	4
Days 9 to 12 of breading	0	1	0	1
Time to mating [a, b, c]	1.10	1.05	1.00	1.20
No. Matings per male [b, c]	1.85	1.95	1.85	1.85
No. Pregnant females	34	38	37	37
No. Pregnancies/No. Mated (per male) [b, c]	0.925	0.975	1.00	1.00
Percent Preimplantation loss (per male) [b, c, d]	2.35	1.73	3.13	2.86
Implants				
Total No.	452	500	464	467
No. /Preg. Female [b, c]	13.5	13.3	12.9	13.2
Resorptions				
Total No.	35	35	31	32
No. Resorptions/No. Implants (litter mean) [b, e]	0.0626	0.0571	0.0621	0.0631
Fetuses				
No. Live	417	465	433	435
No. Live/Preg. Female [b, e]	12.5	12.5	12.1	12.3

[a] Each mated female was assigned a value of 1 through 3 based on which of 3 consecutive 4-day periods the mating occurred.

[b] Expressed as reconverted rankit treatment means.

[c] Value without an asterisk denote no significant difference ($p > 0.05$) from the control based on an analysis of variance.

[d] Percent preimplantation loss = ((no. corpora lutea – no. implants) / no. corpora lutea) x 100

[e] Values without an asterisk denote no significant increase ($p > 0.05$) compared to the control based on an analysis of variance.

3.10.1.4 Study 4

Study reference:

Toxicological and Teratogenic Evaluation of Glycerol Formal, study report from 1981 reported as study001 in IUCLID´s section on developmental toxicity

Detailed study summary and results:

Test type

Similar to OECD TG 414 (Prenatal developmental toxicity study)

GLP statement is given.

Test substance

- Glycerol formal
- Purity: >99%

Test animals

- Female CRCD rats (Charles River)
- 25 pregnant females/dose group, 24 in control group
- Age at study initiation: 12 - 13 weeks
- Weight at study initiation: 217 to 281 g
- Source: Charles River, Wilmington, Massachusetts
- Fasting period before study: not specified
- Housing: Following identification of mating, females were housed in stainless steel cages up to 3 per cage
- Diet: Purina Certified Rodent Chow #5002 ad libitum
- Water: tap water was available ad libitum
- Acclimation period: not specified

- ENVIRONMENTAL CONDITIONS
- Temperature (°C): 72°F
- Humidity (%): not specified
- Air changes (per hr): not specified
- Photoperiod (hrs dark / hrs light): 12/12

Administration/exposure

- Oral via gavage
- Daily from GD 6 - 17
- Doses: 0, 75, 150, 300, 600 mg/kg bw/d
- Control animals treated with water

- Historical control data reported from 4 studies (only for wavy ribs available). No additional details on the historical control data are available in the study report (for example regarding the rat strains used or the date of the experiment etc.)
    - Study A

      No. foetuses examined: 506; No. litters examined: 38; No. malformed foetuses: 14; No. litters with malformations: 6 (16%)
    - Study B

      No. foetuses examined: 496; No. litters examined: 39; No. malformed foetuses: 20; No. litters with malformations: 10 (25%)
    - Study C

      No. foetuses examined: 514; No. litters examined: 39; No. malformed foetuses: 18; No. litters with malformations: 6 (15%)
    - Study D

      No. foetuses examined: 261; No. litters examined: 20; No. malformed foetuses: 6; No. litters with malformations: 6 (30%)
- Vehicle: deionized water
- Amount of vehicle: 5ml/kg bw
- All drug solutions were prepared daily
- Stability and concentration: The stability and concentration of the glycerol formal dosing solutions were confirmed by chemical analysis

Description of test design:

- Impregnation procedure: cohoused with non-exposed males
- Proof of pregnancy: sperm in vaginal smear referred to as day 0 of pregnancy
- Sacrifice of the females on GD20 by cervical dislocation under chloroform anaesthesia
- Observations and tests:
    - Daily observation of general behaviour
    - Maternal body weight – recorded on days GD 0, 6, 8, 10, 12, 14, 16, 18, 20
    - Reproductive status of dams:
        - Number and position of implants
        - Number of resorptions
        - Number of live/dead foetuses
        - Number of corpora lutea
    - Examination of foetuses:
        - External examination (every foetus)

- Visceral examination (every 3rd foetus in each litter and all dead and externally malformed foetuses were examined
- Head examination (heads of foetuses that were given routine visceral examination were fixed in Bouin's solution and examined by freehand serial sections)
- Skeletal examination (every foetus)

- Statistics: Data was examined for homogeneity of variance using the Levene Test and for normality using the Wilk and Shapiro W statistic. Significance at P = 0.05 was based on analysis of variance using a least significant difference procedure; normalizing for nonparametric data when necessary. The average live foetal weight per litter was adjusted for the time of sacrifice by covariance analysis.

Results and discussion

- There were no clinical signs of toxicity among females in any of the treatment groups.
- Glycerol formal had no adverse effect on maternal body weight gain.
- Reproductive status (for all results see Table 7)
  - In the highest dose group (600 mg/kg bw/d) an increase in the total number of resorptions (11/3/6/5/28) was observed. The number of resorptions/number of implants per female was significantly ($P \leq 0.05$) increased (0.03/0.01/0.02/0.02/0.07). The number of live foetuses/pregnant female was significantly ($P \leq 0.05$) decreased (12.8/13.0/12.5/12.4/11.3). The number of live foetuses was decreased (308/313/300/262/288) and the number of dead foetuses increased (0/0/0/2/16).
  - Dose-dependent statistically significant decreases ($P \leq 0.05$) in average foetal weight per litter in foetuses from 150, 300 and 600 mg/kg bw/d groups were observed (3.68/3.69/3.38/3.16/2.18).

Table 7: Reproductive status of females

Dose in mg/kg bw/d	0	75	150	300	600
Females					
Total no.	24	25	25	25	25
No. live pregnant	24	24	24	21	24
No. live not pregnant	0	1	1	4	1
No. died pregnant	0	0	0	0	0
No. died not pregnant	0	0	0	0	0
No. sacrificed pregnant	0	0	0	0	0
No. sacrificed not pregnant	0	0	0	0	0
No. aborted	0	0	0	0	0
Implants					
Total no.	319	316	306	267	316
No. /pregnant female[a]	13.3	13.2	12.8	12.7	13.2
Preimplantation loss[a,b,c]	6.63	4.61	6.76	5.68	4.89

Resorptions					
Total no.	11	3	6	5	28
(No. resorptions/no. implants) per female[a,b]	0.03	0.01	0.02	0.02	0.07*
Foetuses					
Total no.	308	313	300	262	288
No. live	308	313	300	260	272
No. live /pregnant female[a]	12.8	13.0	12.5	12.4	11.3*
No. dead	0	0	0	2	16
(No. dead /no. impl. – no. resorp.) per female[a,b]	0.00	0.00	0.00	0.01	0.04*
No. of males/no. of females[d]	166/142	145/168	157/143	141/121	146/141(1)
Average weight (g)/litter[a,b,e]	3.68	3.69	3.38*	3.16*	2.81*

[a] Value without an asterisk denote no significant difference (P>0.05) from control

[b] Expressed as rankit adjusted mean values

[c] Percent preimplantation loss = ((no. corpora lutea – no. implants)/no corpora lutea) x100) per female

[d] Number in parenthesis represent foetuses of indeterminate sex

[e] Adjusted for time of sacrifice by covariance analysis

- External foetal examination (for all results see Table 8)
    - External malformations in foetuses were increased in the two highest dose groups: Anal atresia (0/0/0/2/7) and tail malformations (0/1/0/4/10) observed in foetuses from dams administrated 300 and 600 mg/kg bw/d, and anasarca in foetuses from the 600 mg/kg bw/d group (0/0/0/0/3+2 in dead foetuses).

Table 8: External foetal examination

Dose in mg/kg bw/d	0	75	150	300	600
No. examined foetuses	308	313	300	260 (2)	272 (15)
- No. with malformations*	0	3	1	7 (0)	13 (2)
- No. of malformations*	0	3	3	9 (0)	21 (2)
- No. with variations*	0	0	0	0 (0)	0 (0)
- No. of variations*	0	0	0	0 (0)	0 (0)
No. of examined litters	24	24	24	21	24
- No. with malformations	0	2	1	6	10
- No. with variations	0	0	0	0	0
Type and number of foetal malformations:					
- Micrognathia	0	0	1	0	0
- Cleft palate	0	0	1	0	0
- Superior ankyloglossia	0	0	1	0	0
- Anasarca	0	0	0	0	3 (2)
- Perineal malformation	0	2	0	3	1
- Atresia ani	0	0	0	2	7
Tail malformation	0	1	0	4	10

Dead foetuses reported in parenthesis

- Visceral foetal examination (for all results see Table 9)

- Visceral malformations in foetuses were observed in the highest dose group: Ventricular septal defects (3/0/2/4/26+6 in dead foetuses) and retroesophageal aortic arch malformations (0/0/0/0/3+1 in dead foetus). In addition, azygous branching variation was observed (0/0/6/6/12).

Table 9: Visceral foetal examination

Dose in mg/kg bw/d	0	75	150	300	600
No. examined foetuses	91	100	93	86 (2)	88 (15)
- No. with malformations	3	0	2	4 (0)	31 (7)
- No. of malformations	4	0	2	4 (0)	44 (10)
- No. with variations	0	1	6	6 (0)	13 (0)
- No. of variations	0	1	6	6 (0)	13 (0)
No. of examined litters	24	24	24	21	24
- No. with malformations	1	0	1	3	16
- No. with variations	0	1	2	5	8
Type and number of foetal alterations					
Left ventricular cavity enlarged (M)	0	0	0	0	1
Right ventricular cavity reduced in size (M)	0	0	0	0	1
Ventricular septal defect (M)	3	0	2	4	26 (6)
Right atrium enlarged (M)	0	0	0	0	2 (0)
Bicuspid valvular malformation	0	0	0	0	1
Pulmonary valvular malformation	0	0	0	0	1
Right-sided aortic arch (M)	0	0	0	0	1 (1)
Enlarged aorta (M)	0	0	0	0	1
Aortic tubular hypoplasia (M)	0	0	0	0	1
Reduced aorta (M)	0	0	0	0	1
Doubled aortic arch (M)	0	0	0	0	1
Retroesophageal aortic arch (M)	0	0	0	0	3 (1)
Reduced pulmonary trunk (M)	0	0	0	0	1
Enlarged pulmonary trunk (M)	0	0	0	0	1
Abnormal origin subclavian artery (M)	1	0	0	0	1 (1)
Tracheal malformation	0	0	0	0	1 (1)
Azygous branching variation	0	0	6	6	12
Subclavian branching variation	0	0	0	0	1
Variation in lung lobation	0	1	0	0	0

Dead foetuses reported in parenthesis

M= malformation, V = variation

- Skeletal foetal examination (for all results see Table 10)
  - Skeletal variations in foetuses were observed dose dependently: wavy ribs (0/3/14/46/62+1 in dead foetus) cervical ribs (3/3/0/7/25+2 in dead foetuses), lumbar rib (35/56/54/56/96), extra lumbar vertebra (0/0/0/16/54).

- A dose-dependent delay in foetal ossification (variation), primarily of the skull bones, vertebra, and sternebrae, in foetuses of all treatment groups was observed: incomplete ossification of skull bones (2/21/41/95/112), incomplete ossified cervical vertebra (0/3/16/86/188), incomplete ossified thoracic vertebra (1/6/26/58/149), incompletely ossified sternebra (73/137/251/244/264), incompletely ossified lumbar vertebra (0/2/6/16/78), incompletely ossified sacral vertebra (0/2/13/18/66), incompletely ossified pelvic bones (1/5/31/82/175).

Table 10: Skeletal foetal examination

Dose in mg/kg bw/d	0	75	150	300	600
No. examined foetuses	308	313	300	260 (1)	272 (11)
- No. with malformations*	0	3	15	46 (0)	66 (2)
- No. of malformations*	0	3	16	46 (0)	67 (3)
- No. with variations	105	177	264	252 (0)	269 (2)
- No. of variations	116	236	451	702 (0)	1239 (2)
No. of examined litters	24	24	24	21	24
- No. with malformations	0	2	8	13	17
- No. with variations	23	24	24	21	24
Type and number of foetal alterations					
Skull bone malformation	0	0	0	0	3
Tympanic bulla malformation	0	0	0	0	1
Scapula malformation	0	0	0	0	0 (2)
Thoracic vertebral malformation	0	0	1	0	0
Hypoplastic rib (M)	0	0	0	0	1
Wavy rib (M)	0	3	14	46	62 (1)
Fused ribs (M)	0	0	1	0	0
Cervical rib (V)	3	3	0	7	25 (2)
Lumbar rib (V)	35	56	54	69	96
Extra lumbar vertebra (V)	0	0	0	16	54
Sternebral variation	0	0	1	0	0
Incomplete ossification skull bone (V)	2	21	41	95	112
Incompletely ossified scapula (V)	0	0	1	0	1
Incompletely ossified cervical vertebra (V)	0	3	16	86	188
Incompletely ossified thoracic vertebra (V)	1	6	26	58	149
Incompletely ossified rib (V)	1	1	5	5	6
Incompletely ossified sternebra (V)	73	137	251	244	264
Incompletely ossified lumbar vertebra (V)	0	2	6	16	78
Incompletely ossified sacral vertebra (V)	0	2	13	18	66
Incompletely ossified pelvic bone (V)	1	5	31	82	175
Incompletely ossified forelimb bones (V)	0	0	0	0	1
Incompletely ossified metacarpal (V)	0	0	4	5	17
Incompletely ossified hindlimb bone (V)	0	0	0	0	1

Incompletely ossified metatarsal (V)	0	0	2	1	6

Dead foetuses reported in parenthesis

*Wavy ribs were considered as malformation by the study authors. According to the DEVTOX database[1] wavy ribs are considered as variation

3.10.1.5 Study 5

Study reference:

Glycerol Formal: Oral Teratogenic Study in the Rat, study report from 1981 reported as study002 in IUCLID´s section on developmental toxicity

Detailed study summary and results:

Test type

Similar to OECD TG 414 (Prenatal developmental toxicity study)

GLP statement is given.

Test substance

- Glycerol formal
- Purity: >99%

Test animals

- Female CRCD rats (Charles River)
- 25 pregnant females/dose group
- Age at study initiation: 12 - 13 weeks
- Weight at study initiation: 217 to 281 g
- Source: Charles River, Wilmington, Massachusetts
- Fasting period before study: not specified
- Housing: Following identification of mating, females were housed in stainless steel cages up to 3 per cage
- Diet: Purina Certified Rodent Chow #5002 ad libitum
- Water: tap water was available ad libitum
- Acclimation period: not specified

- ENVIRONMENTAL CONDITIONS
- Temperature (°C): 72°F
- Humidity (%): not specified

[1] https://www.devtox.org/nomenclature/ml_organ.php?lan=en (Accessed on 1.2.2021)

- Air changes (per hr): not specified
- Photoperiod (hrs dark / hrs light): 12/12

Administration/exposure

- Oral via gavage
- Daily from GD 6 - 17
- Doses: 0, 10 mg/kg bw/d
- Control animals treated with water
- Vehicle: deionized water
- Amount of vehicle: 5ml/kg bw
- All drug solutions were prepared daily
- Stability and concentration: The stability and concentration of the glycerol formal dosing solutions were confirmed by chemical analysis

Description of test design:

- Impregnation procedure: cohoused with non-exposed males
- Proof of pregnancy: sperm in vaginal smear referred to as day 0 of pregnancy
- Sacrifice of the females on GD20 by cervical dislocation under chloroform anaesthesia
- Observations and tests:
    - Daily observation of general behaviour
    - Maternal body weight – recorded on days GD 0, 6, 8, 10, 12, 14, 16, 18, 20
    - Reproductive status of dams:
        - Number and position of implants
        - Number of resorptions
        - Number of live/dead foetuses
        - Number of corpora lutea
    - Examination of foetuses:
        - Foetal weight
        - Skeletal examination (every foetus)

- Statistics: Data was examined for homogeneity of variance using the Levene Test and for normality using the Wilk and Shapiro W statistic. Significance at P = 0.05 was based on analysis of variance using a least significant difference procedure; normalizing for nonparametric data when necessary. The average live foetal weight per litter was adjusted for the time of sacrifice by covariance analysis.

Results and discussion

- There were no clinical signs of toxicity among females.
- Glycerol formal had no adverse effect on maternal body weight gain.
- Reproductive status (for all results see Table 11):

  No effects on the number of implants, resorptions, or live and dead foetuses per litter were observed.

Table 11: Reproductive status of females

Dose in mg/kg bw/d	0	600
Females		
Total no.	25	25
No. live pregnant	24	23
No. live not pregnant	1	1
No. died pregnant	0	0
No. died not pregnant	0	0
No. sacrificed pregnant	0	0
No. sacrificed not pregnant	0	0
No. aborted	0	0
No. sacrificed discarded	0	1
Implants		
Total no.	340	323
No. /pregnant female[b]	14.2	14.0
Resorptions		
Total no.	29	13
(No. resorptions/no. implants) per female[a,b]	0.06	0.03
Foetuses		
Total no.	311	310
No. live	311	310
No. live /pregnant female[a,b]	13.0	13.5
No. dead	0	0
No. of males/no. of females	154/157	155/155
Average weight (g)/litter[a,b,c]	3.60	3.60

[a] Value without an asterisk denote no significant difference (P>0.05) from control

[b] Expressed as rankit adjusted mean values

[c] Adjusted for time of sacrifice by covariance analysis

- Skeletal foetal examination (for all results see Table 12):

  No evidence of teratogenic effects in foetuses administered 10 mg/kg bw/d were observed.

  According to the authors of the study the cojoined twin foetuses in the exposure group were considered to be a spontaneous occurrence unrelated to compound administration. The non-significant (P>0.05) increase in the incidence of lumbar rib in foetuses from the exposure group was also considered a spontaneous occurrence not related to compound administration. Lumbar ribs with a widely variable incidence are observed in the historical controls of the laboratory (0 – 27.8%, no

further information available) and the incidence in the exposure group is well within this range (14.2%).

The study authors also concluded that the increased incidence of incompletely ossified sternebra in the exposure group lies well within the range of values observed in the historical control data (no further information provided).

Table 12: Skeletal foetal examination

Dose in mg/kg bw/d	0	10
Foetuses		
- No. examined	311	310
- No. with malformations*	4	2
- No. of malformations*	4	2
- No. with variations	103	113
- No. of variations	120	141
Litters		
- No. examined	24	23
- No. with foetal malformations	4	2
- No. with foetal variations	22	20
Type and number of foetal alterations[a]		
Sternebral malformation	1	0
Wavy rib (M)	3	0
Hypoplastic rib (M)	0	1
Cojoined twin-craniopagus frontalis	0	1
Incompletely ossified skull bone (V)	18	8
Incompletely ossified atlas (V)	1	0
Incompletely ossified cervical vertebra (V)	0	3
Incompletely ossified sternebrae (V)	54	76
Cervical rib (V)	4	3
Incompletely ossified rib (V)	1	0
Lumbar rib (V)	29	44
Incompletely ossified thoracic vertebra (V)	7	3
Incompletely ossified lumbar vertebra (V)	2	1
Incompletely ossified pelvic bones (V)	3	1
Extra lumbar vertebrae (V)	1	2

[a] M = malformation, V = variation

*Wavy ribs were considered as malformation by the study authors. According to the DEVTOX database[2] wavy ribs are considered as variation

3.10.1.6 Study 6

Study reference:

Aliverti, V.; Bonanomi, L.; Giavini, E.; Leone, V.G.; Mariani, L. (1980), Effects of glycerol formal on embryonic development in the rat, Toxicology and Applied Pharmacology, 56, 93-100 (Aliverti et al., 1980)

2 https://www.devtox.org/nomenclature/ml_organ.php?lan=en (Accessed on 1.2.2021)

Detailed study summary and results:

Test type

Similar to OECD TG 414 (Prenatal developmental toxicity study)

No GLP statement is given.

Test substance

- Glycerol formal
- Purity: 99%

Test animals

- Sprague-Dawley (for experiments 1, 2, 3 and 4); Wistar (for experiment 5) rats
- 10 pregnant females/dose group
- Source: Charles River Italia, Calco, Italy
- Age at study initiation: not specified
- Weight at study initiation: 250 ± 20 g
- Assigned to test groups randomly: yes
- Fasting period before study: not specified
- Housing: Pregnant rats were housed individually in air-conditioned quarters.
- Diet: Commercially available pellets (Altromin MT, obtained from Rieper, Vandoies, Italy) ad libitum
- Water: Water ad libitum
- Acclimation period: 2 weeks

- ENVIRONMENTAL CONDITIONS
- Temperature (°C): 22 ± 2
- Humidity (%): 60 ± 5
- Air changes (per hr): not specified
- Photoperiod (hrs dark / hrs light): 12/12

Administration/exposure

- Administration intramuscular (im); subcutaneous (sc); oral (po)
- Exposure from GD 6 – 15 in experiments 1, 3, 4 and 5 or from GD 6-15, 7-8, 9-10, 11-12, 13-14, 15-16 in experiment 2.
- Sacrifice on gestation day 21

- Doses / Concentrations: 0 mL/kg, 0.25 mL/kg (300 mg/kg) in experiment 1, 0.50 mL/kg (600 mg/kg) in experiments 1, 2, 3, 4 and 5, 1.0 mL/kg (1200 mg/kg) in experiments 1 and 4
- Dose selection rationale: dosages were selected on the basis of previous range-finding studies
- Rationale for animal assignment (if not random): random
- Controls received physiological saline

Description of test design:

- Impregnation procedure: cohoused
- Mating overnight of pairs of female rats with a male of proven fertility of the same strain
- M/F ratio per cage: 1/2
- Length of cohabitation: overnight
- Further mating after two unsuccessful attempts: no
- Verification of same strain and source of both sexes: no
- Proof of pregnancy: sperm in vaginal smear referred to as day 1 of pregnancy
- Any other deviations from standard protocol: no

- The test is divided into 5 experiments.
    - Experiment 1

      Glycerol formal was administrated at 3 dose levels by intramuscular route to Sprague-Dawley rats throughout organogenesis to determine a reference standard dosage.

    - Experiment 2

      Glycerol formal was administrated at the reference standard dosage and at different periods during gestation to determine the day(s) of gestation when the embryo was most sensitive to the teratogenic effects of glycerol formal.

    - Experiment 3

      Embryotoxic and teratogenic effects of glycerol formal administration were investigated by comparing subcutaneous to intramuscular route.

    - Experiment 4

      Embryotoxic and teratogenic effects of glycerol formal administration were investigated by comparing oral to intramuscular route.

    - Experiment 5

Embryotoxic and teratogenic effects of glycerol formal administration by subcutaneous and intramuscular routes were investigated in Wistar rats instead of Sprague-Dawley rats.

- Observations and tests:
  - Maternal observations:
    - BODY WEIGHT: Yes
    - Time schedule for examinations: Gain between Day 1 and 21
    - POST-MORTEM EXAMINATIONS: Yes
    - Sacrifice on gestation day 21
    - Organs examined: Uterus
    - The ovaries and uterine content were examined after termination: Yes
    - Examinations included:
    - Number of implantations: Yes
    - Number of resorptions: Yes
  - Foetal observations:
    - External examinations: Yes: [half per litter]
    - Soft tissue examinations: No
    - Skeletal examinations: Yes: [half per litter]
    - Weight: Yes
    - Head examinations: No
    - For foetuses´ abnormalities, malformation rate was calculated as number of abnormal foetuses divided by number of examined foetuses.
- Statistical analysis of foetal body weight was performed by the analysis of variance. The mean weight of the foetuses from the same litter was established as the independent variable and group means were compared with the Dunnet t-test (Dunnet, 1995).

  The postimplantation loss and the incidence of visceral and skeletal abnormalities were analysed by the chi-square test: differences among groups for total number of live foetuses, resorptions, and dead foetuses (postimplantation loss) were evaluated considering an "n x 3" contingency table whereas differences for total number of normal and abnormal foetuses were evaluated by an "n x 2" contingency table (Kimball, 1951, 1954).

Results (see Table 13, Table 14 and Table 15)

- Maternal toxicity:
  - No maternal toxicity was observed in any of the tests.
- Experiment 1

- Glycerol formal was administered at 3 dose levels via intramuscular application to Sprague-Dawley from GD 6-15
- Postimplantation loss rate increased dose-dependently from 4.4% (control) to 63.7% (highest dose). The mean weight of live foetuses decreased from 4.1 g (control) to 3.2 g (highest dose). Significant differences from control values were demonstrated in all treated groups.
- The number of females with malformed foetuses increased dosed dependently (0/2/6/7). The incidence of visceral malformations increased dose-dependently (malformation rate (MR): 0/0/28/75). Malformations were particularly found in the cardiovascular system. Typical of these malformations was a ventricular septal defect involving a more or less extensive communication between the two ventricules sometimes accompanied by cardiomegaly, atrial hypertrophy, and right and retroesophageal aortic arch.
- Foetuses found dead in the uterus had widespread subcutaneous oedema and ventricular septal defects involving extensive intraventricular communication.
- The malformation rate of skeletal costal defects was 0/15/10/22. Skeletal anomalies were mostly limited to wavy ribs.

- Experiment 2
  - Glycerol formal was administrated at 600 mg/kg at different time periods during gestation to determine the day(s) of gestation when the embryo was most sensitive to the teratogenic effects.
  - In the positive control group (exposed to glycerol formal from GD 6-15), the embryotoxic and teratogenic effects observed in experiment 1 were reproduced. The same treatment for only 2 consecutive days (any period tested) between GD 7 and 16 induced no cardiovascular malformations. Postimplantation loss rates were always significantly lower than the positive control group rate.
- Experiment 3 and 4
  - Embryotoxic and teratogenic effects of glycerol formal administration was investigated by comparing subcutaneous to intramuscular route (exp. 3) and oral to intramuscular route (exp. 4).
  - Administration by subcutaneous and oral routes (GD 6 - 15) led to similar effects compared to those observed after intramuscular administration. Postimplantation loss rates and visceral malformation rates for were even higher after oral exposure
- Experiment 5
  - Embryotoxic and teratogenic effects of glycerol formal administration by subcutaneous and intramuscular routes were investigated in Wistar rats instead of Sprague-Dawley rats.

- Glycerol formal administrated at 0.50 mL/kg either im. or sc. on GD 6 - 15 to Wistar rats induced a lower postimplantation loss rate (but still statistically significant) and a lower frequency of cardiovascular malformations compared to Sprague Dawley rats. However, skeletal costal defects were more frequent in Wistar compared to Sprague-Dawley rats.

Table 13: Influence of glycerol formal on pregnant Sprague-Dawley (SD) or Wistar (W) rats - Maternal data

Experiment No.	Strain*	Group	Dose (ml/kg)	Dose (mg/kg bw/d)	Route**	GD of treatment	Number of females with					Maternal weight gain (g) between Days 1 and 21
							Positive vaginal smears	Implantation signs	Live foetuses	Resorptions	Malformed foetuses	
1	SD	1	0	0	im	6-15	10	9	9	4	0	+ 92
	SD	2	0.25	300	im	6-15	10	8	7	5	2[a]	+ 88
	SD	3	0.50	600	im	6-15	10	8	8	6	6	+ 87
	SD	4	1.00	1200	im	6-15	10	9	7	9	7	+ 95
2	SD	5	0.50	600	im	6-15	7	7	7	7	7	+ 118
	SD	6	0.50	600	im	7+8	9 (1[b])	8	8	5	2[a]	+ 112
	SD	7	0.50	600	im	9+10	9	9	9	1	3[a]	+123
	SD	8	0.50	600	im	11+12	8	8	8	4	0	+ 98
	SD	9	0.50	600	im	13+14	8	7	6	7	1[a]	+121
	SD	10	0.50	600	im	15+16	9	9	9	3	6[a]	+ 140
3	SD	11	0	0	sc	6-15	5	5	5	2	0	+ 126
	SD	12	0.50	600	im	6-15	7 (1[b])	6	5	6	5	+ 133
	SD	13	0.50	600	sc	6-15	9	9	7	9	7	+ 98
4	SD	14	0	0	po	6-15	6	6	6	5	0	+ 88
	SD	15	0.50	600	po	6-15	11	11	8	9	8	+ 113
	SD	16	1.00	1200	po	6-15	8 (2[b])	5	1	5	1	+ 61
5	W	17	0	0	im	6-15	5	5	4	2	1[a]	+ 110
	W	18	0.50	600	im	6-15	13	11	11	7	6	+ 110
	W	19	0.50	600	sc	6-15	7	5	5	3	3	+ 101

* SD: Sprague Dawley, W: Wistar

** im: intramuscular, sc: subcutaneous, po: oral

[a] Skeletal anomalies

[b] Number of females which died in the course of the experiment is reported in parentheses.

Table 14: Influence of glycerol formal on pregnant Sprague-Dawley (SD) or Wistar (W) rats - Litter data

Experiment No.	Strain*	Group	Dose (ml/kg)	Dose (mg/kg bw/d)	Route**	GD of treatment	Implantations number	Resorptions number	Dead foetuses number	Postimplantation loss rate (%)	Live foetuses number	Mean weight (g) live foetuses
1	SD	1	0	0	im	6-15	114	5	0	4.4	109	4.1
	SD	2	0.25	300	im	6-15	94	8	0	8.5[a]	86	3.6[a]
	SD	3	0.50	600	im	6-15	116	17	6	19.8[a]	93	3.1[a]
	SD	4	1.00	1200	im	6-15	127	63	18	63.7[a]	46	3.2[a]
2	SD	5	0.50	600	im	6-15	95	35	10	47.3	50	2.2
	SD	6	0.50	600	im	7+8	103	6	0	5.8[b]	97	3.8[b]
	SD	7	0.50	600	im	9+10	132	1	0	0.7[b]	131	3.6[b]
	SD	8	0.50	600	im	11+12	119	7	0	5.8[b]	112	3.2[b]
	SD	9	0.50	600	im	13+14	78	21	0	26.9[b]	57	3.6[b]
	SD	10	0.50	600	im	15+16	117	5	0	4.2[b]	112	3.7[b]
3	SD	11	0	0	sc	6-15	56	2	0	3.5	54	4.0
	SD	12	0.50	600	im	6-15	80	23	0	28.7[c]	57	3.4[c]
	SD	13	0.50	600	sc	6-15	98	32	3	35.7[c]	63	3.0[c]
4	SD	14	0	0	po	6-15	62	1	0	1.6	61	3.7
	SD	15	0.50	600	po	6-15	127	62	9	55.9[d]	56	3.0[d]
	SD	16	1.00	1200	po	6-15	49	47	0	95.9[d]	2	2.4 ND
5	W	17	0	0	im	6-15	40	2	0	5.0	38	3.8
	W	18	0.50	600	im	6-15	108	15	2	15.7 NS	91	2.9[e]
	W	19	0.50	600	sc	6-15	49	5	0	10.2 NS	44	3.3 NS

* SD: Sprague Dawley, W: Wistar

** im: intramuscular, sc: subcutaneous, po: oral

[a] Statistically different ($p < 0.05$) from group 1 value.

[b] Statistically different ($p < 0.05$) from group 5 value.

[c] Statistically different ($p < 0.05$) from group 11 value.

[d] Statistically different ($p < 0.05$) from group 14 value.

[e] Statistically different ($p < 0.05$) from group 17 value.

ND Statistical significance not determined because of the small number of observations.

NS Non significantly different ($p > 0.05$) from the control value.

Table 15: Influence of glycerol formal on pregnant Sprague-Dawley (SD) or Wistar (W) rats - Incidence of abnormalities

Experiment No.	Strain*	Group	Dose (ml/kg)	Dose (mg/kg bw/d)	Route**	GD of treatment	Abnormalities								
							External			Visceral cardiovascular defects			Skeletal costal defects		
							No. EF[a]	No. AF[b]	MR	No. EF	No. AF	MR	No. EF	No. AF	MR
1	SD	1	0	0	im	6-15	109	0	0	59	0	0	50	0	0
	SD	2	0.25	300	im	6-15	86	0	0	47	0	0 NS	39	6	15[d]
	SD	3	0.50	600	im	6-15	93	1	1	53	15	28[d]	40	4	10[d]
	SD	4	1.00	1200	im	6-15	46	0	0	28	21	75[d]	18	4	22[d]
2	SD	5	0.50	600	im	6-15	50	0	0	25	16	64	25	11	44
	SD	6	0.50	600	im	7+8	97	0	0	58	0	0[e]	39	3	7[e]
	SD	7	0.50	600	im	9+10	131	1	0.7	75	1	1.3[e]	56	5	8.9[e]
	SD	8	0.50	600	im	11+12	112	0	0	64	0	0[e]	48	0	0[e]
	SD	9	0.50	600	im	13+14	57	0	0	32	0	0[e]	25	3	12 NS
	SD	10	0.50	600	im	15+16	112	0	0	59	0	0[e]	53	13	24 NS
3	SD	11	0	0	sc	6-15	54	0	0	29	0	0	25	0	0
	SD	12	0.50	600	im	6-15	57	0	0	34	12	35.3[f]	23	15	65[f]
	SD	13	0.50	600	sc	6-15	63	1	1.5	34	16	47[f]	29	7	24[f]
4	SD	14	0	0	po	6-15	61	0	0	31	0	0	30	0	0
	SD	15	0.50	600	po	6-15	56	2	3.5	27	20	74[g]	29	6	20[g]
	SD	16	1.00	1200	po	6-15	2	0	0	1	1	100 ND	1	0	0 ND
5	W	17	0	0	im	6-15	38	0	0	20	0	0	18	2	11
	W	18	0.50	600	im	6-15	91	1	1	56	5	8.9 NS	35	16	46[h]
	W	19	0.50	600	sc	6-15	44	0	0	23	3	13 NS	21	10	47[h]

* SD: Sprague Dawley, W: Wistar

** im: intramuscular, sc: subcutaneous, po: oral

[a] number of examined foetuses (EF)

[b] number of abnormal foetuses (AF)

[c] malformation rate (MR) in percent

[d] Statistically different ($p < 0.05$) from group 1 value.

[e] Statistically different ($p < 0.05$) from group 5 value.

[f] Statistically different ($p < 0.05$) from group 11 value.

[g] Statistically different ($p < 0.05$) from group 14 value.

[h] Statistically different ($p < 0.05$) from group 17 value.

ND Statistical significance not determined because of the small number of observations.

NS Non significantly different ($p > 0.05$) from the control value.

3.11 Specific target organ toxicity – single exposure

Evaluation not performed for this substance.

3.12 Specific target organ toxicity – repeated exposure

Evaluation not performed for this substance.

3.13 Aspiration hazard

Evaluation not performed for this substance.

4 ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance.

5 REFERENCES

Aliverti, V.; Bonanomi, L.; Giavini, E.; Leone, V.G.; Mariani, L. (1980)
Effects of glycerol formal on embryonic development in the rat
Toxicology and Applied Pharmacology, 56, 93-100