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# Chloroform

CAS #67-66-3

Swiss CD-1 mice, at 0.0, 6.6, 15.9, and 41.2 mg/kg by gavage

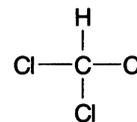
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Chloroform, which is currently used as a chemical intermediate and is found in the very low parts per billion range as a byproduct of chlorine-based drinking water disinfection, was tested for its effects on reproduction and fertility in Swiss CD-1 mice, following the RACB protocol. Data on body weights, clinical signs, and food and water consumption from a 2-week dose-range-finding study (Task 1) were used to set exposure levels for the Task 2 continuous cohabitation phase at 8, 20, and 50 mg/kg/day by gavage. Analysis of the dosing solutions showed that the actual doses administered to the animals were closer to 7, 16, and 41 mg/kg, because of volatilization of the chloroform during preparation and dosing.

Five animals died during Task 2; these deaths were scattered throughout the dose groups and were not judged related to treatment. Food and water consumption was not affected by treatment; group mean body weights during Task 2 differed by no more than 2%.

There were no treatment-related changes in any end point related to reproductive function during Task 2. Dam body weights, number of litters, number of pups per litter were all unchanged. At the end of Task 2, the last litter from all dose groups was reared by the dam until weaning. There were no treatment-related alterations in pup viability or increase in body weight. At weaning, the F<sub>0</sub> mice were killed and discarded without necropsy. Following the protocol of a "negative" study, at weaning the pups from the low and middle dose groups were killed and discarded, and the pups from the control and high dose groups were reared and dosed through the mating period (at approximately postnatal day 74) until necropsy.

Thus, the second generation reproductive assessments were conducted using pups from the control and high dose groups only. Of the 20 cohabited control pairs, only 14 delivered live pups, while 19 of 20 high dose pairs delivered live pups; this difference was significant. The treated pairs

delivered 12 pups per litter, compared to the controls' 10. There were no other differences between the groups.

After the F<sub>2</sub> pups were evaluated and discarded, the F<sub>1</sub> adults were killed and necropsied. While there was no difference in female body weights, female body weight-adjusted liver weight was elevated by 14% in the treated group. In males, the only difference between the groups was a 7% increase in relative epididymis weight. There were no differences between the groups in epididymal sperm measures. Vaginal cytology was not evaluated in these animals.

Treatment-related histologic alterations in males included hepatitis and hepatocellular degeneration (1 case each). All treated females showed some degree of hepatocellular degeneration. No changes were seen in lung, thyroid, spleen, esophagus, or the accessory sex organs.

In summary, chloroform had no adverse effect on mouse reproductive end points at doses that were hepatotoxic.

CHLOROFORM

Summary: NTP Reproductive Assessment by Continuous Breeding Study.

NTIS#: 89148639/AS

Chemical: Chloroform

CAS#: 67-66-3

Mode of exposure: Gavage

Species/strain: Swiss CD-1 mice

F <sub>0</sub> generation	Dose concentration →	7 mg/kg	16 mg/kg	41 mg/kg
General toxicity		Male, female	Male, female	Male, female
Body weight		—, —	—, —	—, —
Kidney weight <sup>a</sup>		•	•	•
Liver weight <sup>a</sup>		•	•	•
Mortality		—, —	—, —	—, —
Feed consumption		•	•	•
Water consumption		—, —	—, —	—, —
Clinical signs		—, —	—, —	—, —

Reproductive toxicity				
$\bar{x}$ litters/pair		—	—	—
# live pups/litter; pup wt./litter		—, —	—, —	—, —
Cumulative days to litter		—	—	—
Absolute testis, epididymis weight <sup>a</sup>		•	•	•
Sex accessory gland weight <sup>a</sup> (prostate, seminal vesicle)		•	•	•
Epidid. sperm parameters (#, motility, morphology)		•	•	•
Estrous cycle length		•	•	•

Determination of affected sex (crossover)		Male	Female	Both
Dose level		•	•	•

F <sub>1</sub> generation	Dose concentration →	•	•	41 mg/kg
General toxicity		Male, female	Male, female	Male, female
Pup growth to weaning		•	•	—, —
Mortality		•	•	—, —
Adult body weight		•	•	—, —
Kidney weight <sup>a</sup>		•	•	—, —
Liver weight <sup>a</sup>		•	•	—, ↑
Feed consumption		•	•	•
Water consumption		•	•	—, —
Clinical signs		•	•	—, —

Reproductive toxicity				
Fertility index		•	•	↑
# live pups/litter; pup wt./litter		•	•	↑, —
Absolute testis, epididymis weight <sup>a</sup>		•	•	—, ↑
Sex accessory gland weight <sup>a</sup> (prostate, seminal vesicle)		•	•	—, —
Epidid. sperm parameters (#, motility, morphology)		•	•	—, —, —
Estrous cycle length		•	•	•

Summary information	
Affected sex?	Unclear
Study confounders:	None
NOAEL reproductive toxicity:	>41.2 mg/kg
NOAEL general toxicity:	Can't be determined
F <sub>1</sub> more sensitive than F <sub>0</sub> ?	Unclear
Postnatal toxicity:	No

Legend: —, no change; •, no observation; ↑ or ↓, statistically significant change (p<0.05); —, —, no change in males or females. <sup>a</sup>Adjusted for body weight.