DATA EVALUATION RECORD

(1) CHEMICAL: Trichlorfon

(2) TYPE OF FORMULATION: "97.85% active material"

(3) CITATION: Rybak, M. 1973. [The effect of Foschlor administered in water on the ontogenetic development of white rats and their progency.] Rocz. Panstw. Zakl. Hig. 24:465-475 (Translated from Polish)

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(6) TOPIC: This study has information pertinent to discipline toxicology, topic reproduction. It relates to the Proposed Guidelines data requirement 163.83-4.

(7) CONCLUSION: Trichlorfon was administered in the drinking water of "white rats" at doses approximating 0.6, 6.0, and 60.0 mg/kg/day. There were no effects seen in the females given 0.6 mg/kg/day, but an insufficient number of both animals and litters per generation at all doses, in addition to the lack of data on stillborns per litter, preclude considering this the no-effect level.

Doses of 6.0 and 60 mg/kg/day resulted in postnatal death, significant ($p<0.05$) in the highest dose group, and tended to reduce the neonatal body weight gain in each of the three generations observed. The lack of data on both the time of postnatal deaths and on maternal toxicity, failure to observe two litters per generation or monitor maternal food consumption, in conjunction with the above insufficiencies, allows this paper to provide only supplementary data for assessing the female reproductive toxicity of trichlorfon.

CORE CLASSIFICATION: Supplementary. An insufficient number of animals per dose were used. No data on either stillbirths per litter or the timing of postnatal death were given.

(8) MATERIALS AND METHODS: "Trichlorfon produced by the 'Azot' Works of Jaworzno [and] containing 97.85% active material," was administered in the drinking water of "white rats," at doses approximating 0, 0.6, 6.0, and 60.0 mg/kg/day

(equivalent to 0.001, 0.01, and 0.1 of the LD_{50}, respectively). Youngs rats, weighing 90-115 g, were divided into 4 groups of 10 females and 3 males each and were called "parents." Females were maintained in groups of five per cage, but no further information was given. Drinking water containing trichlorfon was continuously available for consumption to the parents and the three successive generations during every phase, prior to mating and during mating and lactation.

Three months after the dosing began, female parents and the females of the three subsequent generations were examined for blood cholinesterase activity by the method of Pushkina (1963. Gos. Iz. Med. Lit.). Also after 3 months, vaginal lavage was performed, twice a day, to determine estrous frequency during the 4th month of exposure during each generation.

It is not clear how the actual doses were calculated; the methods section reports that body weight was taken every 2 weeks and that the amount of trichlorfon was measured for calculation of the dose per kg body weight per day. In the results section the body weight and dose calculation were reportedly done daily according to the methods of Cabejszek et al. (Rocz. PZH 15:345). This inconsistency, however, does not alter the interpretation of the data. The author also does not specify whether or not individual

doses were calculated for each rat or if each group of rats was treated as the dosing unit.

Mating began after the 4th month of exposure in each generation that was bred only once; males and females within each group were combined and, after 10 days, the females were placed in individual cages. No further details were given. The number of pups alive and dead were recorded at birth and through 21 postnatal days (F_1 generation). Body weights of surviving neonates were taken during the 3rd and 4th weeks postpartum; weaning occurred at the end of the 4th week. The time to eye opening and the appearance of hair were recorded as were possible anomalies.

Weanlings, weighing close to the mean in each group, were used as progenitors of the subsequent generation while being maintained on the same trichlorfon dosage. Dose groups were comprized of 10 females and 3 males. Both the second and the third generations were produced by the above methods.

Experimental results were statistically evaluated by Student's t-test relative to the control group within each generation. A significance level of $p<0.05$ was chosen.

(9) REPORTED RESULTS: The actual average doses of trichlorfon that each group received are presented in the following table. The authors did not state what the figures on range of variations of each group represent.

Target dose (mg/kg/day)	Parents	Generation F1	F2	F3
0.6	0.6±0.07	0.7±0.09	0.6±0.1	0.7±0.07
6.0	7.2±0.7	7.6±0.7	7.4±0.9	8.2±0.8
60.0	63.5±8.4	67.8±5.2	69.5±11.9	63.5±10.4

Blood cholinesterase activity (percentage of control) was significantly reduced, in a dose-related fashion, in parents and each of the three generations at doses of 6.0 and 60 mg/kg/day. The second generation given 0.6 mg/kg/day also exhibited a significant reduction in blood cholinesterase activity. Neither the number of analyses averaged per group nor the standard deviation are given for this data, but there was a 4-fold variation in mean values among the four generations treated with the highest dose, and a 2-fold variation among control generations and other dose groups.

The frequency of estrus was not altered by trichlorfon from the 3 to 4 day occurrence in controls (The author stated on page 7, "3-4 weeks"--This is concluded to be a misprint or translation error based on the insignificant difference reported between the 3.5 to 4 day estrous frequency of the control and the dosed females.).

There were no significant reductions in either the frequency of conception or the length of gestation. The

number of rats born in the third generation given 60 mg/kg/day was greatly reduced (38 pups in 5 liters versus 76 pups in 8 control litters); this reduction, however, is reflective of the significant neonatal mortality occurring at 60 mg/kg/day in the previous generation. The following table shows the percentage of "mortality of young rats in relation to the general number of births":

	Generation		
Target Dose	1	2	3
Control	10.8%	15.8%	7.8%
0.6	23.1%	8.1%	14.1%
6.0	6.5%	37.2%*	31.1%
60.0	57.9%*	73.8%*	78.9%*

*Significant at $p<0.05$

The author attributed the high neonatal mortality to a lack of feed for the newborns, which "was confirmed in a great many mothers." No further explanation was given, except that these effects were not observed in groups given 0.6 or 6.0 mg/kg/day.

In all generations, there was no deviation in either the time to hairing or eye opening; no external anomalies were observed. Neonates from the second and third generations in the 60 mg/kg/day groups exhibited, according to the author, "significant differences in the body weight increase

...during the first four weeks," which were not statistically significant in "the later period." The figure in the paper delineates the body weight graphically by week, but a statistical significance for weeks 3 and 4 cannot be extricated from the graphs.

(10) DISCUSSION: The study offers substantiation for the effects of trichlorfon on reproduction, but falls short of discerning the extent or true nature of those effects. It is clear that postnatal viability of the pups is affected by both 6.0 and 60 mg/kg/day, but the lack of data on the number of stillborns per litter, the postnatal period most frequently affected, and maternal toxicity precludes assessing the primary cause of this postnatal loss. The author's reference to high neonatal mortality being caused by newborns receiving too little feed may be unclear in the original or may have been obscured by translation.

The effects of trichlorfon on male reproduction are also inadequately assessed. Although the lowest frequency of conception was in the control group, using three males offers only suggestive evidence for the absence of an effect.

The rationale is not stated in the paper, but determining blood cholinesterase levels in the females of a reproductive study can be considered only an indicator for the consumption of the dosing substance, especially

in this study, where no information on the number of animals assayed or the relative error of the measurements were given.

What remains is 6.0 mg/kg/day as the lowest effective dose for female reproduction.

(11) TECHNICAL REVIEW TIME: 7.5 hours