DATA EVALUATION RECORD

(1) CHEMICAL: Trichlorfon

(2) TYPE OF FORMULATION: 97.85% Active ingredient

(3) CITATION: Wojcik, J. 1975. The effect of Foschlor in drinking water on the internal organs of three successive generations of rats. Rocz. Panstw. Zakl. Hig. 26:383-391 (Translated from Polish)

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(6) TOPIC: This study has information pertinent to discipline toxicology, topic subacute effects. It relates to the Proposed Guidelines data requirement 163.82-1.

(7) CONCLUSION: Trichlorfon was given in their drinking water to female Wistar rats, at doses approximating 0, 0.6, 6.0, and 60 mg/kg/day, for 6 months to each of three successive generations. The study of Rybak (1973. Rocz. Panstw. Zakl. Hig. 24:465-475, DER 32B-0060) was carried out simultaneously and provides additional information and data.

Body weight tended to decrease with dose, but was significant ($p<0.05$, Student's t-test) only at 60 mg/kg/day in the second generation. Spleen weight tended to increase, but was significantly greater only at 60 mg/kg/day in the third generation. Histologically, liver parenchyma showed swelling and hyperchromatic nuclei with scattered cell lysis; these were most "intense" at 60 mg/kg/day.

Results for the second and third generation given 60 mg/kg/day are of limited value in that a discrepancy exists in the number of females used. Lack of adequate definition prevents data for liver histochemistry from delineating a per-generation or generation-to-generation effect and lack of statistical analysis obscures the significance of the data for urinary 17-ketosteroids.

These data augment the toxicity profile of trichlorfon with 6.0 mg/kg/day as a no-effect level in Wistar rats for changes in the parameters evaluated in this study.

CORE CLASSIFICATION: Supplementary. No males were evaluated and no food consumption data were presented.

(8) MATERIALS AND METHODS: Data for this study were collected from animals used concurrently to assess the reproductive effects of Trichlorfon for three generations (Rybak 1973).

Trichlorfon from the "AZOT" works in Jaworzno, Poland, containing 97.85% active ingredient, was mixed with the drinking water of Wistar rats ("of our own breeding") to approximate daily doses of 0.6, 6.0, and 60 mg/kg. The actual dose each of the three generations received is shown in the following table:

Target Doses (mg/kg/day)	Generation		
	P_1	P_2	P_3
0.6	0.6±0.07	0.7±.09	0.6±0.1
6.0	7.2±0.7	7.6±0.7	7.4±0.9
60.0	63.5±8.4	67.8±5.2	69.5±11.9

Each group contained 10 female rats continuously dosed for 6 months and fed a processed diet containing 28.5% oats, 19% rye, 19% wheat, 14% meat-bone meal, 7% dry fodder yeast, 5% powdered milk, 5% casein, 1% cod liver oil, 1% kitchen salt, and 0.5% calcium carbonate. "The LSM granulated feed was mixed with the [processed] diet in a ratio of 1:2."

No explanation of the origin of each generation used in this study was given, but is available from Rybak (1973) and is as follows: The parent generation, not used by Wojcik, consisted of 10 females and 3 males per dose group.

After 4 months of dosing, males and females from the same dose group were combined for 10 days (no further information available); the females were then removed to individual cages. Litters born were called the first generation (P_1) and, after 4 weeks, pups weighing close to the mean body weight for each dose group were selected to produce the next generation (P_2). The third generation was produced by the same protocol with trichlorfon-dosed drinking water available continuously through each phase of each generation.

Each generation was sacrificed, after 6 months of dosing, by decapitation and the internal organs (liver, kidney, suprarenal glands, spleen, ovaries, and hypophysis) were examined grossly, weighed, and prepared for histological staining (hemotoxylin-eosin). Liver cuttings were analyzed, by "routine methods employed in histochemistry," for fat, glycogen, RNA, alkaline phosphatase, nonspecific oxidases, and respiratory enzymes (succinate dehydrogenase and cytochrome oxidase). "The thickness of the individual layers of the suprarenal cortex was then measured with the aid of a micrometer under a microscope." In addition to the foregoing postmortem observations, rats in P_2 and P_3 were assessed for levels of 17-ketosteroids in the urine, which was collected daily.

It is assumed that the body weight was measured as reported by Rybak (1973) "every two weeks."

Statistical analysis was by Student's t-test, but no fiducial limit for signficance was given.

(9) REPORTED RESULTS: Body weight, averaged for the 10 rats in each group, decreased at 60 mg/kg/day, but was only significant in the P_2 generation; it is not clear what length of time is included in each average body weight calculation.

Individual organ weights were not affected relative to the control values, except for the spleen weights in the P_3 generation, which were increased at 6.0 and, significantly, at 60 mg/kg/day.

Morphometric measurement of the suprarenal cortex showed discernable changes in rats given 60 mg/kg/day; the reticular layer "underwent slight shrinkage (approximately 11%); however the glomular [sic] and banded layers were softened." Similar conditions were observed in the individual layers of the cortex in the P_2 and P_3 generations; none of the changes in any generation was significant and numerical data were presented only for the P_1 generation. These changes were not reflected in the organ weight data for the suprarenal gland.

Rats receiving trichlorfon consumed, approximately, 15% less fluid than controls and also eliminated less urine than control rats; no data were given for the volume of dose consumed.

As shown in the following table, the average daily urine volume and excretion of 17-ketosteroids in the urine of P_2 and P_3 generation rats are both decreased at 6.0 and 60.0 mg/kg/day. No standard error or significant difference was given.

Generation	Target Dose	Concentration of 17-Ketosteroids in Urine ($\mu g/cm^3$)	Daily Urine Volume (cm^3)
P_2	0	1.96	10.1
	0.6	2.10	9.8
	6.0	1.86	7.6
	60.0	1.81	7.5
P_3	0	2.2	9.2
	0.6	1.95	11.2
	6.0	1.80	8.6
	60.0	1.78	8.4

Histopathological changes of the liver parenchyma were "most intense" at 60 mg/kg/day and were characterized by overall cell enlargement and hyperchromatic nuclei, "disintegrated cells were sporadically visible" and "[h]isto-chemical changes made up the morphological change in the parenchymal cells." Histochemically, the liver showed the following changes:

Target Dose (mg/kg/day)	Quantity of Trichlorfon LD50	Fat	Glycogen	RNA	DH	OC	FK	FZ	EN
0.6	0.001	-	-	-	-	-	-	-	-
6.0	0.01	±	-	±	±	±	±	-	±
60.0	0.1	+	+	+	++	++	+	-	+
0	Control	-	-	-	-	-	-	-	-

++ = pronounced changes
+ = weak changes
± = residual changes
- = no changes
RNA = ribonucleic acid
DH = succinate dehydrogenase
OC = cytochrome oxidase
FK = acid phosphatase
FZ = alkaline phosphatase
EN = nonspecific esterases

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There was also a "low degree of steatosis" (fatty degeneration) observed in the liver sections. These data are not clearly defined as being from a single generation or an "average" of the three generations.

(10) DISCUSSION: There are several shortcomings in this paper, which limit the value of the given data. (a) The translation may have obscured several areas, particularly the description of the changes observed in the suprarenal gland, as well as the extent of the relationship between this study and that of Rybak (1973). (b) The protocol for recruiting each generation is not explained or clearly referenced. If the same animals used by Rybak were used for this study, the 60 mg/kg/day-group in P_2 contained 5 females and P_3 also contained less than 10 females (38 pups born, but 78.9% died, leaving only 8 neonates in P_3; Rybak 1973).

Wojcik reported all data as averages of 10 rats per dose per generation; the data for the highest dose (60 mg/kg/day) are of questionable validity. (c) Histochemical results from liver preparations are not identified as being from a single generation or from an average of the three. None the less, the effect of 60 mg/kg/day is apparent on the hepatic enzymes assayed, but neither the "per generation" nor generation-to-generation effect is discernable. (d) The significance of the excretion of 17-ketosteroids is obscured by the absence of standard deviations or statistical analysis. (e) No reference or relative standard was given for the differentiation of one suprarenal cortical zone from the next into which it gradually transits. Furthermore, measuring histologically prepared tissues ignores the normal vagaries induced by the preparative process; 11% variation would not seem to be dose related. (f) The author did not address the 15% reduction in fluid consumption of the trichlorfon-dosed rats from the aspect of taste aversion or toxicity. This may be related to the body weight reduction by either dehydration or toxic anorexia; in either case, the body weight changes were not reflected in the organ weights. Food consumption data would have been beneficial.

(11) TECHNICAL REVIEW TIME: 11.5 hours