Acta vet. scand. 1983, *24*, 295—304.

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TERATOGENIC EFFECTS OF THE ORGANOPHOSPHORUS COMPOUND FENCHLORPHOS IN RABBITS

By

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NAFSTAD, I., G. BERGE, E. SANNES and A. LYNGSET: *Teratogenic effects of the organophosphorus compound fenchlorphos in rabbits.* Acta vet. scand. 1983, *24*, 295—304. — Fenchlorphos was administered orally in doses of 0, 12.5, 25 and 50 mg per kg to pregnant rabbits from day 6 to 18 of gestation. No effect on implantation efficacy, number of live fetuses, or fetal weight was observed. The incidence of major malformations such as cardiovascular and brain anomalies was, however, increased in the medicated groups. Major skeletal malformations were more frequent in the medicated groups; minor skeletal variation were about equal in all groups. Dose-relationship was observed for cardiovascular malformations and cerebellar hypoplasia.

fenchlorphos; teratogenicity; rabbits.

Fenchlorphos was tested in a reproduction study in pregnant blue foxes and was found highly embryotoxic and teratogenic in a dosage commonly used for the treatment of sarcoptic mange (*Berge & Nafstad* 1983). Other organophosphorus compounds, such as trichlorphon, have produced cerebellar hypoplasia in piglets when used for the treatment of sarcoptic mange in pregnant sows (*Kronevi* 1977, *Knox et al.* 1978). We therefore decided to investigate the possible teratogenic potency of fenchlorphos in a laboratory animal species, and the Belted Dutch rabbit was chosen as the experimental animal.

MATERIALS AND METHODS

Test material

Fenchlorphos (0.0.-dimethyl-0-(2,4,5-trichlorophenyl)phosphorthioate) was obtained as the farmaceutical preparation Ectoral® from Pitman-Moore, Inc., USA.

Animals and housing

Nulliparous female Belted Dutch rabbits of a local stock were used. The animals were 16—18 weeks of age, weighed 1.6—2.8 kg and were of a conventional hygienic quality. Prior to the experiment they were tested serologically with the india ink immunoreaction test for Encephalitozoon cuniculi-infection (*Waller et al.* 1979) and found negative. The bucks used for semen donation were Belted Dutch rabbits of the same local strain as the does aged 4—12 months.

The animals were housed separately in metal cages measuring 45×45×38 cm equipped with automatic watering system. They were fed ad libitum with a commercial pelleted breeding diet for rabbits and guinea pigs (Ewos, Södertälje, Sweden). Room temperature was 20—22°C, relative humidity 40—60 %, and light-darkness cycle 12:12 h.

Experimental design

Artificial insemination was performed according to *Lenz* (1976) with the modification described by *Lyngset & Sannes* (1979). The day of insemination was designated as day 0 of gestation. Fenchlorphos was administered orally in doses of 0, 12.5, 25 and 50 mg per kg body weight from day 6 to 18 of gestation. The tablets were powdered in an electrical grinder and mixed with lactose (1:10).

The daily dose for each individual doe was calculated on the basis of the body weight on day 6, and was kept the same level throughout the experiment. The dose mixture was administered to the individual rabbit by stomach tube after mixing with distilled water. The total amount of water was 10 ml per rabbit. The control rabbits were given the corresponding amount of lactose in distilled water.

Blood samples for the analysis of cholinesterase activity were drawn by a vacutainer from the central ear artery in amounts of 3—5 ml on days 6, 8 and 28 of gestation.

Each animal was weighed weekly on the same week day. The experiment was performed during April and May.

Determination of cholinesterase activity

Acetylcholinesterase activity was measured according to the radiochemical method of *Sterri & Fonnum* (1978). Ten ml of enzyme solution were mixed with 10 ml of a substrate consisting of 2.6 mmol/l (I-^{14}C) acetylcholine at a concentration of 0.49 μCi/μmol, 0.2 mmol/l ethopropazine, and 20 mmol/l sodium phosphate pH 7.4. The mixture was incubated at 30°C for 15 min, and then transferred to a scintillation vial and diluted with 1 ml of 0.1 mol/l sodium phosphate buffer pH 7.4, to terminate the reaction. The labelled acetate was extracted by the addition of 10 ml Insta-Fluor scintillation mixture (Packard Instrument Company) and 4 ml of 0.2 mol/l trioctylamamonium phosphate in iso-amyl alcohol, and by light shaking for 1—2 min. Liquid scintillation counting was carried out with a Packard Tri-Carb 3310 spectrometer and the results were expressed as counts per min and calculated individually in % of the corresponding value 2 days before the medication started. Butyrylcholinesterase was determined with the same method except that ethopropazine was omitted from the substrate.

Necropsy and teratological examination

The does were killed by a blow on the head on day 29 of gestation and gross examination of all organs was performed. The ovaries were examined for the presence and number of corpora lutea. The uteri were examined with respect to number of implantation sites and live and dead fetuses. The live fetuses were sacrificed by an i.p.injection of 0.5 ml pentobarbitone (10 %), weighed and sexed. The heads were fixed in 10 % neutral formalin and examined by the razor blade technique of *Wilson* (1965). One section from the cerebellum area from 10 animals per group was embedded in paraffin for histological examination. The viscera were examined by gross inspection, and samples from the liver and kidney were collected for histological examination. The carcasses of all fetuses were fixed in 70 % ethanol and subjected to the alizarin staining method of *Dawson* (1926) for the examination of skeletal abnormalities. The samples for histological examination were prepared according to standard techniques and stained with hematoxylin and eosin.

Statistical evaluation

The litter was considered the experimental unit for analysis of data regarding embryotoxicity and teratogenicity. Average percentages were calculated for each examination technique according to the formula: 100 x (No. of malformed fetuses in the litter/total number of fetuses examined in the litter/total number of litters), as described by *Marks et al.* (1981). Pairwise comparisons of group means to the vehicle control group mean were calculated by the Fisher-Irwin significance test (*Hodges & Lehman* 1970).

RESULTS

Reproduction performance

All the does remained clinically healthy throughout the experimental period. The reproduction data are presented in Table 1. There were no significant differences between groups with regard to number of litters produced, number of implan-

Table 1. Effect of fenchlorphos on reproduction in rabbits.[a]

	Control[b]	Dose (mg/kg/day)		
		12.5	25.0	50.0
Number of pregnant does examined	14	16	11	13
Number of litters with live fetuses	11	15	11	13
Average (± s) weight gain during gestation (days 6—29) (g)[c]	132±72	181±71	268±51	365±45
Average (± s) uterus weight (g)	81±5	84±6	89±4	75±5
Average number of implantation sites[d]	6.7	6.2	6.6	6.0
Average number of live fetuses pr. litter	4.1	4.4	5.7	5.1
Pre-implantation loss (%)	19.8	23.9	8.4	17.6
Post-implantation loss (%)[e]	33.9	25.8	12.6	18.7
Average (± s) fetal weight (g)[f]	30.4±1.4	33.3±1.5	36.1±1.4	29.7±1.6
Female/male live fetuses	31/27	36/35	32/31	32/34

[a] Killed on day 29 of gestation after receiving fenchlorphos on days 6—18

[b] Received vehicle on days 6—18

[c] Include the fetuses in pregnant animals. The lower weights indicate that some abortions have occurred

[d] Per pregnant female

[e] Includes resorptions, abortions and dead fetuses

[f] Dead and aborted fetuses were excluded

tation sites, number of live fetuses per litter, or number of post implantation losses. The average fetal weight and the female to male ratio were about equal for all groups.

Teratogenicity

The results of the teratological examination are presented in Table 2. The percentage of litters with malformed fetuses and the average percent of malformed fetuses per group showed higher values for the fenchlorphos groups than for the control group.

Table 2. Teratogenic effects of fenchlorphos on rabbit fetuses.[a]

	Control	Dose (mg/kg/day)		
		12.5	25.5	50.0
Number of fetuses examined	58	71	63	66
Percentage of litters with malformed fetuses[b]	45	73	73	85
Average % malformed fetuses[b,c]	11	46*	24	41*
Number of malformations per malformed fetus[b]	1.5	1.5	1.5	1.6
Average % fetuses with malformations in the cardiovascular system[c]	10	18	19	40*
Average fetuses with malformations in the CNS[c,d]	8	15	2	12
Average % fetuses with malformations in the eyes[c]	0	1	0	2
Average % fetuses with major malformations in the skeletal system[c]	0	8	2	7
Average % fetuses with minor skeletal variations[c]	45	39	51	50
Average % fetuses with histological changes in the cerebellum[c,e]	0	0	45	54
Average % fetuses with miscellaneous malformations[c]	0	4[f]	3[g]	2[h]

[a] Dead fetuses were excluded from all malformation computations
[b] Minor skeletal variations and histological changes in the cerebellum not included
[c] Formula described in Materials and Methods
[d] Histological changes in the cerebellum not included
[e] Ten fetuses were examined in each group
[f] Three fetuses with gastrocele
[g] Two fetuses with ovarial ectopia/hypoplasia
[h] One fetus with cleft palate and one with ovarial ectopia/hypoplasia
* Statistically significantly higher than the control group (Fisher-Irwin $p = 0.05$)

The types of CNS and eye malformations included varying degree of dilated cerebral ventricles, external hydrocephalus, microcephaly, exencephaly, and microphtalmy. The cardiovascular abnormalities were aortic arch hypoplasia, aortic and pulmonary stenosis, septal defects, and common aortal-pulmonary trunk. The skeletal abnormalities were divided in major malformations and minor variations. The former group included syndactylia, micromelia, amelia, and fused or split ribs; the latter group included variations in the number of ribs and vertebrae, and delayed ossification of bones.

Gastroschisis, cleft palate, and hypoplasia of the gonads occurred sporadically.

No clear dose relationship was found for the total number of malformations, CNS and eye abnormalities or skeletal malformations. Cardiovascular abnormalities and histologically detectable hypoplasia of the cerebellar cortex showed however a tendency towards dose-effect relationship. For the lastmentioned abnormality, there was no difference between the control and the low dose group, while in the medium dose group six out of 10 fetuses showed indistinct foliation and hypocellularity of the internal granular layer of the cerebellar cortex (Figs. 1 and 2). Similar changes were present in all the 10 examined fetuses in the high dose group.

No significant lesions were observed by histological examination of the liver and the kidney.

Table 3. Cholinesterase activity (mean ± s) in erythrocytes and plasma of pregnant does administered fenchlorphos on days 6—18 of gestation.

Cholinesterase activity	Day of gestation	Control	Dose (mg/kg/day)		
			12.5	25.0	50.0
Acetylcholinesterase activity* (Erythrocytes)	8	99±16	79±15	88±16	74±19
	28	106±18	87±14	80±15	77±20
Butyrylcholinesterase activity* (Plasma)	8	99±16	72±18	66±15	54±15
	28	106±18	76±19	64±14	61±19

* The activity was calculated in percent of the individual value measured on day 6 of gestation (2 days prior to medication) which was set to 100 %.

Cholinesterase activity

The results of the cholinesterase activity determinations are presented in Table 3. None of the does developed a clinically significant degree of cholinesterase inhibition. The control animals showed a tendency towards increment in the cholinesterase activity from the start to the end of gestation. In the medicated groups, the activity either remained unchanged or decreased slightly during gestation. On comparing the enzyme activity in blood samples collected on day 28 of pregnancy, a trend towards dose-associated decrease could be seen. The difference between groups seems to be minor when the average values of groups are compared. By the comparison of individual does which produced litters with 2 or more fetuses with major malformations with the average values of the group, the decrease in cholinesterase activity was more obvious. Examples of such individuals are shown in Table 4.

Table 4. Acetylcholinesterase activity in erythrocytes of individual does administered fenchlorphos on days 6—18 of pregnancy as compared with average activity of the corresponding groups. Individuals with 2 or more malformed fetuses are selected.

	Day of gestation	Dose group 25 mg/kg				Dose group 50 mg/kg			
		Number of doe[1]			Average for whole group	Number of doe[1]			Average for whole group
		38(3)	39(2)	43(4)		5(3)	30(5)	72(5)	
Acetylcholinesterase activity[2]	8	84	72	90	88	80	93	82	74
	28	61	63	74	80	71	66	40	77

[1] Number of fetuses with major malformations in the litter is given in parenthesis.

[2] Acetylcholinesterase activity was calculated as percent of the individual value measured at day 6 of gestation, which was designated 100 %.

DISCUSSION

In contrast to our findings in the teratology experiment with fenchlorphos in blue foxes (*Berge & Nafstad* 1983), the present experiment gave no indication of an adverse effect of fenchlorphos on the fertility in Belted Dutch rabbits. In the blue fox, there was a severe reduction of the number of live whelps, while in the rabbits this effect was not observed. However, the results

from the 2 experiments are not directly comparable, because different doses of fenchlorphos were used. Our primary intention was to compare the effect of the same dose level in the 2 species. However, preliminary testing of the dose regimen showed that our rabbit stock did not tolerate 100 mg/kg, which was used for the foxes. In the rabbits, severe toxicity symptoms occurred as a consequense of an approximate 90 % inhibition of cholinesterase with this dose.

The relatively high incidence of post-implantation losses which appeared in the present experiment needs some comments. A post-implantation loss of up to 30 % is higher than normal in our Belted Dutch rabbit stock. There was no indication that fenchlorphos was a causal factor for this finding, since the incidence was similar in the control and the medicated groups. A possible explantation for the increased incidence of post-implantation losses could be that the application technique with the passing of a stomach tube daily during the administration period represented a rather heavy stress factor.

The results of the present experiment indicate a teratogenic potential of fenchlorphos in the Belted Dutch rabbit, as reflected by the increased incidence of congenital malformations in the medicated groups, and the tendency towards dose relationship for some types of abnormalities. In previous investigations in piglets cerebellar hypoplasia has occurred following therapeutic use of trichlorfon in pregnant sows (*Kronevi* 1977, *Knox et al.* 1978, and others). We found that similar lesions developed in blue fox whelps after the administration of therapeutic doses of fenchlorphos to pregnant vixens (*Berge & Nafstad* 1983). In the blue fox whelps, there was also an increased incidence of major malformations in other organs. Similar teratogenic effects of

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Figure 1. Comparison of control rabbit fetus (A) and rabbit fetus from the high dose group (50 mg fenchlorphos per kilo daily on days 6—18 of pregnancy, B). Cerebellar hypoplasia with indistinct foliation and hypocellularity of internal granular layer (IGL). EGL = external granular layer, ML = molecular layer. H & E, × 170.

Figure 2. Greater magnification of cerebellar cortex from rabbit fetus in the high dose group (B in Fig. 1). Note scarcity of cells in internal granular layer and no visible Purkinje cells. H & E, × 425.

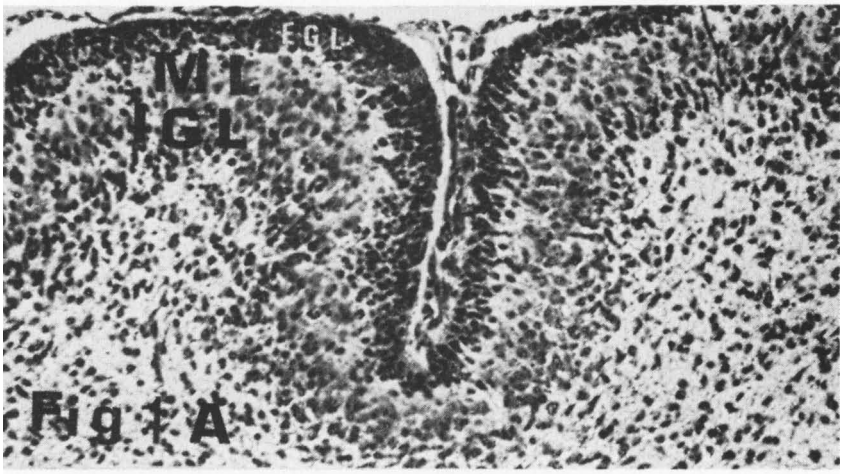

Fig 1A

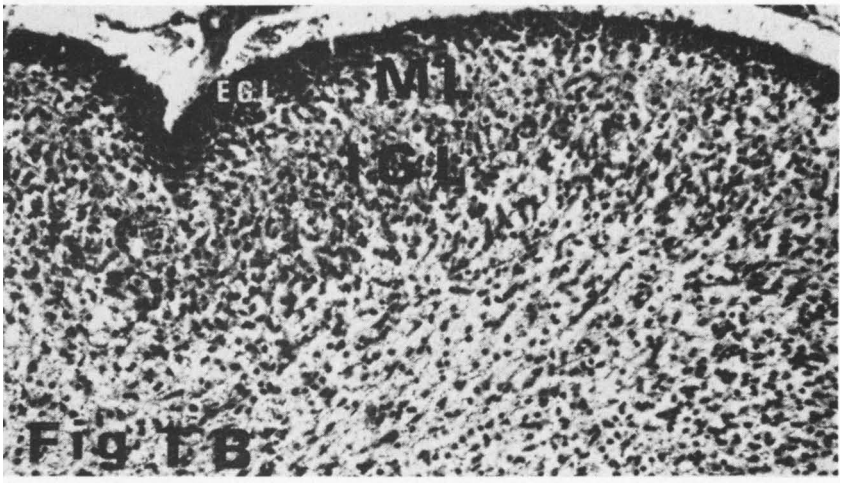

Fig 1B

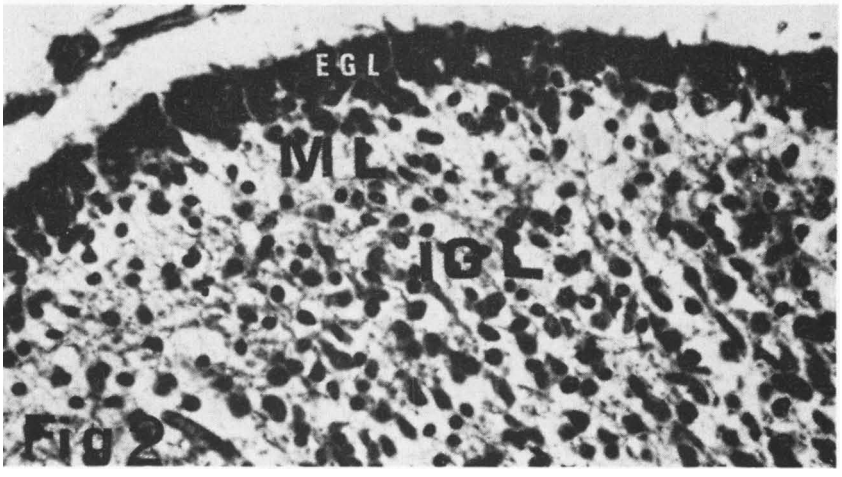

Fig 2

fenchlorphos were not observed in a teratogenicity study performed in rats (*Khera et al.* 1981). In this rat experiment, histological examination of the brain was, though, not carried out.

In the present experiment, there seemed to be a dose-related incidence of cerebellar hypoplasia. The no-effect level dose for cerebellar hypoplasia appeared to be 12.5 mg/kg body weight.

ACKNOWLEDGEMENTS

The excellent technical assistance given by Chief Animal Technician Per Solberg is gratefully acknowledged. The authors wish to express their sincere thanks to Ms. Mette Førde for performing the analyses of cholinesterase activity.

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SAMMENDRAG

Teratogen effekt av fosforinsektisidet fenklorfos på kanin.

Ektoparasittmiddelet fenklorfos (0.0-dimethyl-0-(2,4,5-triklorofenyl)fosforthioat) ble administrert i doser på 0, 12.5, 25 og 50 mg per kg til drektige Belted Dutch kaniner fra 6. til 18. drektighetsdag. Behandlingen hadde ingen effekt på implantasjonstall, antall levende foster, fostervekt, eller kjønnsfordeling hos fostrene.

Det var forøket antall misdannede fostre i de doserte gruppene sammenlignet med kontrollgruppene. Misdannelsene omfattet hjertekar, hjerne og skjelett.

Histologisk undersøkelse av cerebellum viste hypoplasi av cerebellarcortex i høyeste og nest høyeste, men ikke i laveste dosegruppe.

(Received August 15, 1983).

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