Acta vet. scand. 1983, *24*, 99—112.

From the Department of Pharmacology and Toxicology, Veterinary College of Norway, Oslo.

TERATOGENICITY AND EMBRYOTOXICITY OF ORALLY ADMINISTERED FENCHLORPHOS IN BLUE FOXES*

By

Gunnar N. Berge and *Inger Nafstad*

BERGE, G. N. and I. NAFSTAD: *Teratogenicity and embryotoxicity of orally administered fenchlorphos in blue foxes.* Acta vet. scand. 1983, *24*, 99—112. — Pregnant blue foxes (Alopex lagopus) were administered fenchlorphos (0-0-dimethyl-0-(2,4,5-trichlorophenyl) phosphorothioate) orally at a dose of 100 mg/kg/day in different periods of gestation. The dose chosen represents the therapeutic dose for the treatment of parasitic lesions. At term the mean number of whelps were recorded, and they were killed and examined for external, visceral and skeletal malformations. Of 19 medicated vixens the mean number of live whelps at term was 1.2 per vixen versus 9.5 in the control group. There was an evident predominance of males in the medicated groups. Several malformations of the head were registered, among them incomplete ossification of the skull bones, cleft palate, hydrocephalus internus and externus. Minor malformations like extra ribs or missing ribs occurred in the medicated groups. Congenital alopecia, hypoplastic kidneys, and hydronephrosis were observed in all the whelps in 1 medicated group. No significant difference in total brain weight, cerebellum weight or the cerebellum-to-total-brain weight was observed.

Histological examination of the cerebellum showed a narrowing or absence of the granular and the molecular layers of the cortical zone.

teratogenicity; embryotoxicity; fenchlorphos; blue fox.

Fenchlorphos is the common name of an organophosphorus compound which has been assigned to 0,0-dimethyl-0-(2,4,5-trichlorophenyl) phosphorothioate of the American Standards Association. This substance is the active ingredient of many

* This work was supported by grants from the Norwegian Agricultural Research Council and The Norwegian Furbreederes Association.

insecticide preparations and is commercially available under different trade names (Ronnel®, Karlan®, Trolene®, Ectoral®). Fenchlorphos has been shown to be highly effective as a systemic insecticide for cattle grub control, as a residiual spray for the control of flies, roaches, screw worms and other parasites. A number of veterinarians have found these preparations to be effective against demodectic mange in dogs (*Lawrence* 1960, *Burch & Brinkman* 1962, *Baker et al.* 1976).

Sarcoptes canis infestation in the blue fox and silver fox in Scandinavia represents a practical problem with respect to treatment, because the animals are kept in outdoor cages, which make dipping and spraying hazardous during winter time. *Søli et al.* (1977) examined the toxicity of systemically administered fenchlorphos as measured by its cholinesterase inhibiting effect, with the aim of establishing an alternative method of treatment. It was concluded that fenchlorphos administration as a feed additive recommended for dogs, 100 mg/kg, is well tolerated by healthy foxes as far as cholinesterase inhibition is concerned. Further studies were carried out by *Berge* (1980) to investigate the clinical effect of fenchlorphos on sarcoptic mange in blue foxes. By the feeding of 100 mg/kg during a periode of 3—5 weeks, the therapeutic effect on sarcoptic mange was considered acceptable.

The present study was designed to evaluate the possible effect of fenchlorphos on reproduction in the blue fox and, additionally, to investigate the embryotoxic and teratogenic potential of the compound.

MATERIAL AND METHODS

Chemical

The organophosphorus insecticide fenchlorphos, 0.0-dimethyl-0-(2.4.5-trichlorophenyl) phosphorothioate, was used in the form of Ectoral® tablets (250 mg) produced by Pitman-Moore, Inc., USA.

Animals and housing

The blue foxes (Alopex lagopus) used in the medicated groups consisted of 15 parous females aged from 2—3 years and 4 1-year old vixens. The control-group was composed of 6 parous females aged from 2—3 years. All the animals were housed indi-

vidually in outdoor fox cages before and during the investigation in the State Research Farm for Furbearing Animals, Heggedal. They were given standard Norwegian wet feed and water ad libitum.

The vixens were mated twice, generally on the 2nd and the 4th day of sexual receptivity. The last day of mating was considered as day 0 of gestation. Oestrus (defnied as the period in which the vixens were willing to mate) usually lasted at least 3 or 4 days.

Experimental design

The experimental animals were divided into 4 medicated groups. Four animals were employed in Group I and 5 animals in each of Groups II, III and IV. Each fox in the experimental groups was fed fenchlorphos at a dose of 100 mg/kg once a day. The tablets were crushed into powder with an electric homogenizer, weighed and divided into daily portions. Due to the unpleasant smell of the substance, it had to be well mixed with the individual feed-portion before administration. Individual food consumption was checked after 1 h. Fenchlorphos was generally taken well when this procedure was used. The animals were observed daily during their gestation periods for general health condition.

Since there is little information available in the literature about the exact critical period for teratogenic effects in the blue fox, and since certain organophosphorus compounds applied to other species during gestation have shown adverse side-effects on reproduction, the compound in this study was administered for varying lengths of time. The foxes in Groups I, II and III (14 females) were medicated for 21 days during gestation. Group I was additionally treated for a period prior to mating, varying from 3 to 20 days before day 0 of gestation. The exact appearance of oestrus in foxes is not possible to predict in advance. The medication of the 4 animals in Group I lasted until day 21 of gestation. The animals in Group II were fed fenchlorphos for 21 days during midpregnancy, and the animals in Group III during the last third of gestation. The vixens in Group IV were given the compound for an average of 50 days, which represent the mean duration of pregnancy in the blue fox. In order to check the conception in the presumptive pregnant females, the foxes were examined by palpation. Blood samples were collected from

the cephalic vein into heparinized tubes before and during gestation for the purpose of analysing the inhibition of the enzymes acetyl- and plasmacholinesterases.

At term the total number of live and dead whelps were recorded. The whelps were weighed, sexed and examined for external malformations. In order to investigate possible cholinesterase-depression in the offspring, the whelps were sacrificed by decapitation and blood samples were collected. The bodies were eviscerated, skinned, cleared with 1 % KOH and stained with the alizarin Red-S method for the examination of skeletal alteration (*Dawson* 1926). The viscera were examined by gross inspection. The emphasis of the post mortem investigation was on a detailed examination of the head. The brain was separated from the spinal cord and weighed. The cerebellum was cut off through the cerebellar peduncles and weighed, and the cerebellum-to-total-brain weight ratio was calculated (*Harding et al.* 1966). One half of the cerebrum and the cerebellum was immersed in 10 % formalin for histological examination and the other half was frozen for determination of cholinesterase activity.

The samples for histological examination were embedded in paraffin, sectioned and stained with hematoxylin and eosin.

The vixens in the trial which did not deliver whelps, were ovario-hysterectomized through a midline incision under Xylazin (Rompun®) anaesthesia within 2 weeks after estimated full term. The uterus and the ovaries were removed and implantation sites were counted.

RESULTS

Most of the animals consumed the feed mixed with fenchlorphos well, but some of the vixens revealed temporary anorexia 3—4 days after the start of medication. The anorexia lasted for a couple of days before the appetite was normalized. Administration of fenchlorphos produced a significant effect on the number of live whelps at term. Of 19 vixens in the medicated groups, only 4 delivered full born whelps, and 1 of these vixens killed her whelps during the first day. The 6 animals in the control group all delivered live whelps. The mean number of whelps was 1.2 per vixen in the experimental animals and 9.5 whelps in the controls, respectively. The average number of live whelps per animal in Groups I, II, III and IV was 0.8, 2.4, 1.4

Table 1. Experimental design and reproduction data from blue foxes given fenchlorphos (100 mg/kg) during pregnancy.

Group	Number of days with fenchlorphos administration	Period of pregnancy for medication	Pregnant/ not pregnant	Number of live whelps	Number of dead whelps	Number of implantation sites	Average number of live whelps
I	25	Prior to mating and early pregnancy	—	0	0	0	0.8
	28		—	0	0	0	
	31		+	3	0	NE	
	40		—	0	0	0	
II	21	Mid-pregnancy	+	0	3[b]	NE	2.4
	21		+	0	0	13	
	21		+	4	4	NE	
	21		+	8	1	NE	
	21		+	0	0	2	
III	21	Late pregnancy	+	0	1	NE	1.4
	21		+	0	0	8	
	21		+	7[a]	0	NE	
	21		+	0	0	10	
	21		+	0	0	1	
IV	50	Whole pregnancy	+	0	0	3	0
	48		+	0	0	9	
	50		+	0	0	4	
	50		+	0	0	1	
	52		+	0	3	NE	
Controls	0		+	11	4	NE	9.5
	0		+	13	0	NE	
	0		+	10	0	NE	
	0		+	7	3	NE	
	0		+	8	2	NE	
	0		+	8	1	NE	

[a] The whelps were killed by the mother.
[b] The birth was induced by an injection of 0.2 ml Estrumate®.
NE Not examined.

Table 2. Summary of reproduction data in blue foxes after daily oral administration of fenchlorphos (100 mg/kg) during pregnancy.

	Experimental Groups	Controls
Number of vixens	19	6
Number of litters	4 (21 %)	6 (100 %)
Average number of live whelps per vixen	1.2	9.5
Average number of dead whelps per vixen	0.6	1.7
Male/female ratio of live whelps	6:1	1.1:1

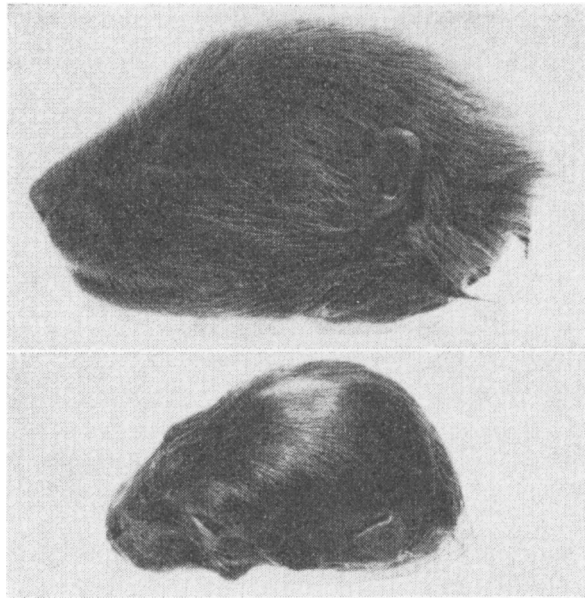
Table 3. Effects of fenchlorphos on the incidence of malformations.

	Experimental Groups				Controls
	I	II	III	total (%)	
Number of whelps examined	3	17	3	23	13
Head deformities					
Incomplete ossification of the skull bones	1	8	3	12 (52 %)	
Peaked snout	1	8	3	12 (52 %)	
Cleft palate		3	1	4 (17 %)	
Outer ear deformity		1		1 (4 %)	
Brain edema/haemorrhages	3	12	3	18 (78 %)	
Indistinct pattern of gyri and sulci of cerebrum	1	8	3	12 (52 %)	
Hydrocephalus externus		2	3	5 (22 %)	
Hydrocephalus internus		5		5 (22 %)	
Malformations in the skeletal systems					
Extra ribs/missing ribs (uni/bilateral)	3	10	1	14 (61 %)	1 (8 %)
Shortened ribs (last rib)	1	10	1	12 (52 %)	
Rudimentary ribs right side		2		2 (9 %)	
Missing lumbar corpora		2		2 (9 %)	
Rudimentary 3. and 4. phalanx			1	1 (4 %)	
Miscellaneous					
Congenital alopecia			3	3 (13 %)	
Hydronephrosis			3	3 (13 %)	
Hypoplastic kidney (uni/bilateral)			3	3 (13 %)	

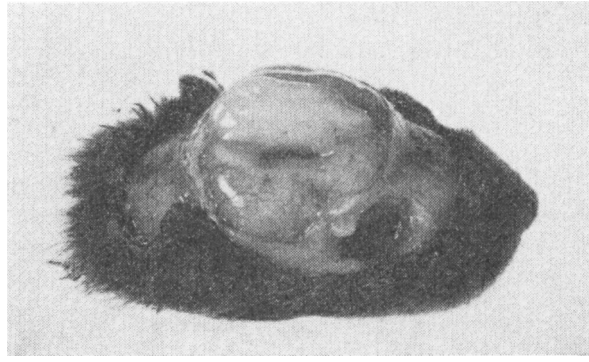
and 0, respectively. The reproduction data are given in Tables 1 and 2.

There was also an evident predominance of male whelps in the medicated groups. The ratio of male to female live whelps was 6:1 in the treated groups compared to 1.1:1 in the controls. Of the 12 ovario-hysterectomized vixens, which did not deliver live or dead whelps at term, implantation sites were observed in 9 animals.

The examination of the head showed a significantly increased occurrence of various malformations in the treated groups (Table 3). Twelve (52 %) of the 23 examined whelps had a pronounced peaked snout (Fig. 1) and incomplete ossification of the skull bones and 1 whelp had malformation of the external ear (Fig. 1). Four whelps (17 %) had cleft palate and hydrocephalus internus (Fig. 2) and hydrocephalus externus was registered in 5 whelps (22 %). Considerable brain edema and menin-



F i g u r e 1. Two whelps from fenchlorphos-treated mother. Abnormal outer ear (top) and peaked snout (bottom).



F i g u r e 2. Brain edema and haemorrhages in whelp from fenchlorphos-treated mother.

geal haemorhages (Fig. 3) occurred in 18 whelps (78 %). The gyri of the cerebral hemispheres were also markedly planed and the consistence of the brain tissue softer than normal. It was not possible to discern between gyri and sulci of the cerebrum in 12 whelps (52 %). None of these defects were observed in control animals.

Significantly increased occurrence of several minor skeletal varians were seen in the medicated groups, including extra/mis-

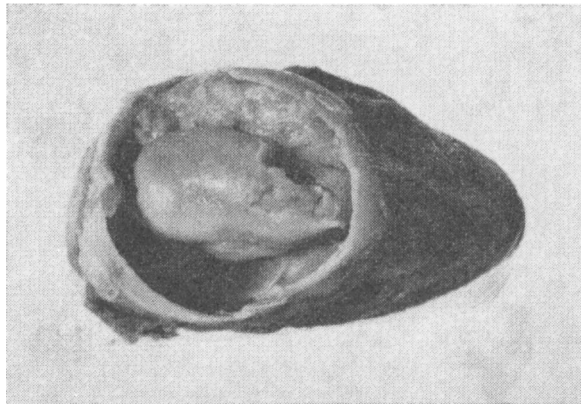


Figure 3. Hydrocephalus internus in whelp from fenchlorphos-treated mother. A piece of the right cerebral hemisphere has been removed to show the dilated lateral ventricle.

sing ribs, shortened ribs, rudimentary ribs right side, missing lumbal corpora. Rudimentary third and fourth phalanx on the left fore limb occurred in 1 whelp in Group IV. Congenital alopecia, hypoplastic kidneys and hydronephrosis were observed in all the whelps in Group IV.

No significant differences in total brain weight, cerebellum weight or the cerebellum-to-total-brain weight was observed (Table 4).

Table 4. Cerebellum-to-total-brain weight ratio in fox whelps from vixens treated with fenchlorphos (100 mg/kg) during pregnancy.

	Experimental Groups	Controls
Number of whelps examined	14	13
Average weight of the whelps (g)	61.63	61.45
Average wegiht of total brain (g)	2.89±0.24	2.98±0.54
Average weight of cerebellum (g)	0.10±0.01	0.10±0.02
Cerebellum/total brain weight ratio	0.04	0.04

The results of the cholinesterase analyses will be published separately.

Microscopical examination

No significant lesions were observed in the cerebrum except edema and haemorrhages in the meninges. The flattening of the

cerebral gyri were appearently caused by extensive meningeal edema in some animals.

The most striking changes were those of the cerebellar hemispheres, consisting of either a narrowing or an almost complete absence of the granular and the molecular layers of the cortical zone; in some cases there was also an incomplete folial pattern (Fig. 4). The hypoplastic areas of the cortical layer interchanged with areas of nearly normal structures. Very few Purkinje cells were seen in the hypoplastic areas.

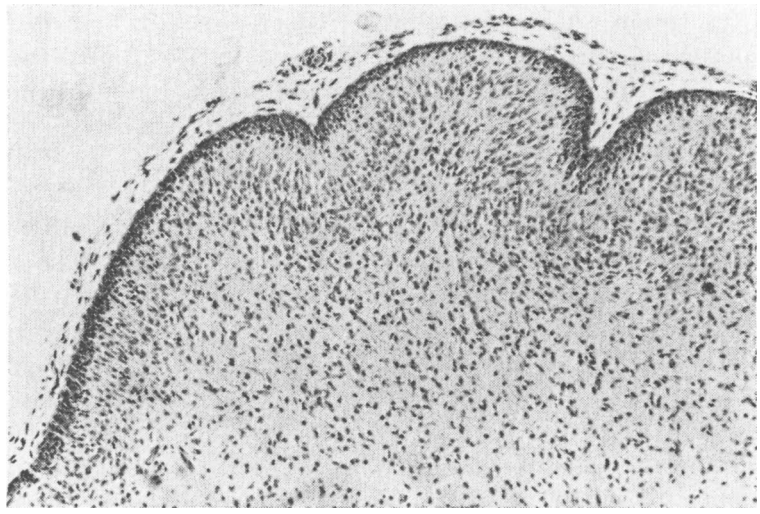


Figure 4. Cerebellum, fox whelp from fenchlorphos-treated vixen. Note meningeal edema and haemorrhages, and incomplete foliation. Narrowing of the cortical layers is seen to the left. HeE × 170.

DISCUSSION

This initial study of the organophosphorus compound fenchlorphos administered orally at a dose of 100 mg/kg/day to blue foxes, implies an embryotoxic and teratogenic potential. Administration of a suitable dosage of a teratogen generally results in the production of some normal offspring, some malformed offspring and some dead or resorbed offspring. The results of this experiment confirm these connections. Only 21 % of the vixens in the medicated groups delivered live whelps, versus 100 % in the control group. This is a significantly decreased incidence of whelps in the species blue fox according to reproduction investigations (*Johansson* 1941, *Sele* 1963, *Christiansen*

1979). *Christiansen* (1979) found that on average 86.7 % of parous blue fox delivered live whelps. A similar percentage has also been registered by other investigators (*Aamdahl & Fougner* 1973). Of the 15 vixens in Groups II—IV, which were given fenchlorphos after the time of implantation, all produced pregnancy, either by delivered live or dead whelps or by the formation of implantation sites in the uterus. Twelve of the vixens did not deliver live whelps, which indicate a considerable postimplantation loss. It is well known that drugs which produce teratogenic disturbances in mammals are likely to produce fetal death and resorption when given at higher doses. It is also known that the dosage of a given teratogen lies within a narrow zone between that which will kill the fetus and that which has no discernible effect. The embryo has a threshold level above which irreparable changes occur, resulting in malformation or death.

The dose administered in the present experiment (100 mg/kg) was the same as that found to be tolerated by adult blue foxes for therapeutic purpose (*Søli et al.* 1977). This dose was tolerated in the vixens in this experiment, even though a temporary anorexia was observed in some animals. Maternal toxicity was not observed apart from the transient anorexia, which is known to occur after administration of cholinesterase inhibitors.

Implantation sites in the uterus resulting from placenta zonaria in the blue fox, can be recorded after day 17 of gestation (*Fougner* 1972). Of 12 ovario-hysterectomized foxes in the medicated groups 9 showed implantation sites. The 3 foxes where implantation sites could not be detected, were all from Group I. This group, however, was medicated prior to mating and during the time of implantation. It is known that a large number of drugs when given to female animals prior to mating and/or gestation, have an antifertility effect by blocking the ovulation (*Schardein* 1976). A possible ability of fenchlorphos to interfere with fertilization must therefore be considered.

The critical period of organogenesis varies among different species and is partly dependent upon the length of gestation. The critical period in the blue fox is not exactly known. However, the duration of pregnancy is 50—53 days; an estimated critical period seemed therefore to include more than the period of medication to Group I. The average number of whelps in all the medicated groups was significantly decreased. No defined

periods of gestation could therefore be identified as especially sensitive.

Several external and internal malformations occurred in the whelps after chronic feeding of fenchlorphos to the vixens during pregnancy. Major malformations such as cleft palate, hydrocephalus internus and externus, and hydronephrosis confirmed these observations.

Minor abnormalities, such as extra thoracic ribs are commonly observed deviations that often occur in mice, rat and rabbits. *Khera et al.* (1981) observed a dose-related increase in the incidence of extra-ribs in a teratogenicity study of fenchlorphos in pregnant rats. The interpretation of these deviations has been subject to some controversy. The variations are regarded as malformations in some works and as spontaneous deviations or indicators of delayed skeletal maturation in others. The literature does not refer to the appearance of these variations in the species blue fox, but it is reasonable to regard the significant increase in these skeletal variations as early signs of teratogenicity.

An interesting observation in this experiment is the histological alteration in the cerebellum of the examined whelps. Several publications in recent years report cases of cerebellum hypoplasia and congenital tremor in piglets after oral application of the organophosphorus compound trichlorphon to sows during different time of gestation (*Bølske et al.* 1978, *Knox et al.* 1978, *Fatzer et al.* 1981).

By histological examination of cerebellum from piglets, *Fatzer et al.* (1981) found that the granular layer of the cerebellum was missing and that there was a hypoplasia of the cerebellar cortex. These findings correspond to the alterations of the cerebellum in the blue foxes found in this experiment after administration of fenchlorphos. It is not known whether a common mechanism exists for the induction of cerebellar hypoplasia after application of organophosphorus compounds, but it is reasonable to suppose that a possible pathogenic mechanism is a repression of cells of the cortical layers in a particular developmental phase. A corresponding reduction of the cerebellum-to-whole-brain ratio, which occurred in piglets after oral administration of trichlorphon, was not observed in the fox whelps following application of fenchlorphos. However, it must be emphasized that the growth and differentiation of the cerebellum normally continue

post partum, which means that the possibility to detect a reduction in cerebellar weight depends on the postnatal time of examination.

It is well known that cerebellar hypoplasia in different animal species can result from viral infections. Examples are felinc enteritis, hog cholera, and bovine virus diarrhoea.

Further, a recent investigation demonstrated that neonatal administration with the antiviral agent cytarabine produced cerebellar hypoplasia in rats (*Gough et al.* 1982).

A common pathogenic mechanism for virus infection, organophosphate toxicity, and antiproliferative activity of certain cytostatic drugs in affecting the external layers of the cerebellum in the pre- and post-natal period could be associated with a high affinity of all compounds for rapidly proliferating cells.

ACKNOWLEDGEMENTS

The authors wish to express their sincere thanks to Dr. med. vet. Ordin M. Møller and Cand. med. vet. Adrian Smith of the State Research Farm for Furbearing Animals for valuable advise and support, and to Mr. S. Dalum and Mr. R. Haugen for excellent technical assistance.

REFERENCES

Aamdahl, J. & J. A. Fougner: Fruktbarhet i blårevavlen (Fertility in the blue fox). Nord. Vet.-Med. 1973, *25*, 504—508.

Berge, G.: Behandling av skabb hos blårev (Treatment of mange in the blue fox). Norsk Vet.-T. 1981, *93*, 6.

Baker, B. B., A. A. Stannard, S. G. Yaskulski & D. C. Hirsh: Evaluation of topical application of Ronnel solution for generalized demodicosis in dogs. J. Amer. vet. med. Ass. 1976, *168*, 1105—1107.

Burch, G. R. & D. C. Brinkman: Systemic insecticide therapy in small animals. Small Anim. Clin. 1962, *2*, 21—27.

Bølske, G., T. Kronevi & N. O. Lindgren: Congenital tremor in pigs in Sweden. Nord. Vet.-Med. 1978, *30*, 534—537.

Christiansen, I. J.: Reproduktion og reproduktionsproblemer hos ræv (Reproduction and reproductive problems in the fox). Dansk Vet.-T. 1979, *62*, 670—680.

Dawson, A. B.: A note on the staining of the skeleton of cleared specimens with Alizarin Red S. Stain Technol. 1926, *1*, 123—124.

Fatzer, R., H. Häni & E. Scholl: Kongenitaler Tremor und zerebelläre Hypoplasie bei Ferkeln nach Behandlung der Mutterschweine mit Neguvon® während der Trächtigkeit. (Congenital tremor and cerebellar hypoplasia in piglets after treatment of mothers with Neguvon® during pregnancy). Schweiz. Arch. Tierheilk. 1981, *123*, 29—36.

Fougner, J. A.: Fosterdød hos blårev (Embryonic death in the blue fox). Nord. Vet.-Med. 1972, *24*, 25—31.

Gough, A. W., N. J. Barsoum, C. J. Difonzo, S. I. Gracon & J. M. Sturgess: Comparison of the neonatal toxicity of two antiviral agents: Vidarabine phosphate and cytarabine. Toxicol. appl. Pharmacol. 1982, *66*, 143—152.

Harding, J. D. J., J. T. Done & J. H. Derbyshire: Congenital tremors in piglets and their relation to swine fever. Vet. Rec. 1966, *79*, 388—390.

Johansson, J.: Oestrus and mating in the silver fox. Ann. Agric. Coll. Sweden 1941, *9*, 239—263.

Khera, K. S., C. Whalen & G. Angers: Teratogenicity study on pyrethrins and rotenon and ronnel in pregnant rats. Teratology 1981, *23*, 45—46.

Knox, B., J. Askaa, A. Basse, V. Bitsch, M. Eskildsen, M. Mandrup, H. E. Ottosen, E. Øverby, K. B. Pedersen & F. Rasmussen: Congenital ataxia and tremor with cerebellar hypoplasia in piglets borne to sows treated with Neguvon® vet. during pregnancy. Nord. Vet.-Med. 1978, *30*, 538—545.

Lawrence, W. A.: Dog tick control with an orally administered acaricide. Mod. vet. Pract. 1960, *41*, 70—71.

Schardein, J. L.: Drugs as Teratogens. CRC Press, Ins., Ohio, USA 1976, Ch. 3, p. 35.

Sele, K.: Parring og avlsresultater for blårev (Fertility in a blue fox farm). Norsk Pelsdyrbl. 1963, *37*, 93—98.

Søli, N. E., R. A. Andersen, J. U. Skaare & A. Mikalsen: The effect of 0,0-dimethyl-0-(2,4,5-trichlorophenyl) phosphorothioate (fenchlorphos) on cholinesterases in the blue fox (Alopex lagopus). Acta vet. scand. 1977, *18*, 408—415.

SAMMENDRAG

Teratogenitet og embryotoksisitet av fenklorfos etter oral administrasjon til blårev.

Fenklorfos (0-0-dimetyl-0-(2,4,5-triklorofenyl)fosforothioat), 100 mg/kg/dag, ble administrert oralt til drektige blårever (Alopex lagopus) i ulike deler av drektighetsperioden. Doseringen som ble valgt, representerer den terapeutiske dose ved behandling av Sarcoptes skabb hos blårev. Ved fødsel ble antall valper registrert, avlivet og undersøkt for eventuelle misdannelser. Av 19 rever i forsøksgruppene ble det født gjennomsnitlig 1,2 valper pr. dyr. Tilsvarende tall i kontrollgruppa var 9,5 valper. Det var en signifikant overvekt av hannvalper i forsøksgruppene. Misdannelser og defekter i form av ufullstendig ossifikasjon av hodeknoklene, ganespalte, hydrocephalus internus og hydrocephalus eksternus ble registrert hos forsøksdyrene. Ulike mindre defekter som overtallige/undertallige ribben ble observert i forsøksgruppene. Kongenital alopeci, hypoplastiske nyrer og

hydronefrose ble registrert hos alle valpene i en forsøksgruppe. Ingen signifikant forskjell mellom gruppene ble registrert med hensyn til vekten av hjernen, cerebellum eller forholdet mellom cerebellum- og hjernevekt. Histologiske undersøkelser viste hypoplasi av granular- og molekularlaget i lillehjernebarken.

(Received January 19, 1983).

Reprints may be requested from: Gunnar N. Berge, the Department of Pharmacology and Toxicology, Veterinary College of Norway, P.O. Box 8146, Dep., Oslo 1, Norway.