Bull. Environ. Contam. Toxicol. (1993) 51:625–632

Environmental Contamination and Toxicology

Developmental Toxicity of Haloxyfop Ethoxyethyl Ester in the Rat

K. Machera

Laboratory of Pesticide Toxicology, Benaki Phytopathological Institute, 8 Stephanou Delta Street, GR-145 61 Kifissia, Athens, Greece

An increasing number of structurally unrelated chemicals of great economic importance, when administered to various mammalian species act in a way similar to hypolipidemic drugs by affecting cholesterol and lipid levels in the blood. So far potential drugs for the treatment of atherosclerosis and related diseases in man, industrial chemicals like plasticizers and agricultural chemicals like phenoxy herbicides have been considered as hypolipidemic agents (Lalwani et al. 1983; Moody and Reddy 1978; Stott 1988). Teratological and embryolethal effects of hypolipidemic compounds on mammals have been reported. These studies were mainly performed with potential drugs, such as M&B 30227 [R,S[4-(2-hydroxy-ethyl)-piperazin-1-yl]-(4-isopropyl- phenyl)-(isothiazol-5-yl)methane dihydrochloride] and M&B 31426 [R,S-(3,5-dimethyl-isoxazol-4-yl)-(4-iso propylphenyl)-(4 methyl-piperazin-1-yl)-methane dihydrochloride] which exhibited embryolethal and teratogenic effects when tested on rats (Steele et al. 1983). Teratogenicity and embryolethality were also observed in rats after the administration of AY 9944 [trans-1,4 bis (2-dichloro-benzyl aminoethyl) cyclohexane dihydrochloride] (Carpent and Desclin 1968; Roux and Aubry 1966; Roux et al. 1972) and triparanol [1-(p-(2 dithylaminoethoxy)phenyl)-1-(p-tolyl-2-(chlorophenyl) ethanol] (Roux 1964; Roux et al. 1973). The most characteristic effects observed in the previous studies were fetal mortality, holopros-encephalies and uro-genital abnormalities. However no teratogenic or embryolethal effects were observed when the hypolipidemic compound MG.46 [4-hydroxy-N-dimethyl-butyramide 4-chlorophenoxy-isobutyrate] was administered to rats, rabbits or mice (Da Lage et al. 1972).

This work is part of a project aimed to study the developmental toxicity of phenoxy herbicides. In this report, the developmental toxicity of the herbicide haloxyfop ethoxyethyl ester on Wistar rats is presented.

Send reprint requests to K. Machera at the above address.

MATERIALS AND METHODS

The test compound haloxyfop ethoxyethyl ester was of technical grade of purity (98.2%) and kindly provided by DOW Chemical Company. The compound was dissolved in carboxy methyl cellulose and administered to the experimental animals at doses 5, 10 and 50 mg/kg body weight in a volume of 10 mL/kg. The same volume of the carrier was administered to the controls.

Female Wistar rats weighing about 200g, fed with a standard diet, were allowed to mate with males of the same stock overnight and vaginal smears were examined in the following morning for spermatozoa. The day on which spermatozoa were found was taken as the first day of gestation. The females were randomly distributed in four groups, one control and three experimental. The test compound was daily administered by gavage to pregnant rats from the 6th to the 16th day of gestation. The animals were weighed daily and examined for general condition and behaviour. They were sacrificed on the 21st day of gestation and the number of implantations, live and dead fetuses and resorption sites were recorded.

The fetuses were weighed, examined under a dissecting microscope and half of them were fixed in Bouin's fluid for the examination of soft tissues. The rest were fixed in 10% ethanol for skeleton examinations. The bodies were dissected according to the technique of Barrow and Taylor (1969). Heads were sectioned and skeletons were prepared and examined according to the technique of Wilson (1965). The data were analyzed by ANOVA followed by Duncan's multiple range comparison procedure.

RESULTS AND DISCUSSION

The effects of the test compound haloxyfop ethoxyethyl ester on the parameters of pregnancy i.e. number of implantation sites, number of live fetuses, number of dead fetuses, number of resorption sites per litter and mean fetal body weight are presented in Table 1. The administration of 10 and 50 mg/kg of the test compound increased the number of resorptions per litter whereas the number of live fetuses per litter was decreased. The incidence of both effects was statistically significant when compared to the control animals. At the dose of 50 mg/kg a statistically significant increase in the number of dead fetuses per litter was observed. Vaginal haemorrhage of various degrees was observed from the 15th day of gestation among the females of the groups treated with 10 and 50 mg/kg with frequencies of 40 % and 70 % respectively. Occurrences of vaginal haemorrhage were not observed among the control animals. In the group treated with 50 mg/kg a statistically significant reduction in

Table 1. Effects of haloxyfop ethoxyethyl ester on the parameters of pregnancy in the rat (means ± SD).

Effects	Haloxyfop ethoxyethyl ester (mg/kg)			
	0	5	10	50
No. of litters	10	10	10	10
No. total implants per litter	11.1±1.3	10.6±1.3	9.1±1.5	9.2±2.2
No. live fetuses per litter	10.9±1.4	10.0±1.5	7.4±1.8[2]	6.4±2.9[2]
No. dead fetuses per litter	0.03±0.0[a]	0.03±0.0[a]	0.3±0.5	1.1±1.4[1]
No. resorptions per litter	0.2±0.4	0.6±0.8	1.4±1.1[1]	1.7±1.5[1]
Fetal body weight (g)	4.7±0.4	4.7±0.6	4.5±0.7	3.9±0.7[1]

[1,2] : Significantly different from the control group for $P<0.01$ and $P<0.001$ respectively

[a] : Values taken from the historical control data of the colony.

the mean fetal body weight was observed when compared to the control animals. Signs of maternal toxicity, such as increased mortality, signs of intoxication, or reduction in body weight were not observed in any of the treated animals.

The effects of the test compound on fetal development as they can be evaluated by the external examination and by the soft tissue examination of fetuses, are summarized in Table 2. A dose depended increase in the number of cachectic fetuses was observed. Cachectic fetuses were developed at a frequency of 2.0, 6.8 and 20.3 % at the doses of 5, 10 and 50 mg/kg, respectively. Ureterohydronephrosis was the most frequently observed malformation at dose depended frequency and severity. At the dose of 50 mg/kg a bilateral dilatation of renal pelvis combined with severe dilatation, bends and sinuosities of ureters were observed in 54.8 % of the examined fetuses. At the dose of 10 mg/kg the abnormality of the ureters was of moderate degree and observed in the 42.9 % of fetuses. About 50 % of the affected fetuses had unilateral, left ureter dilatation, bends and sinuosities, combined with dilatation of renal pelvis. At the dose of 5 mg/kg mild, not statistically significant, ureterohydronephrosis was observed. In this case a mild left ureter dilatation was the only symptom observed. A statistically significant increase in ureterohydronephrosis in the groups treated with 10 and 50 mg/kg was observed. Severe edema, absent tail, open nasal cavity, absence of lower jaw, unilateral microphthalmia, deformed rear appendages and kinky tails were the malformations observed in the treated animals. The incidence of these malformations was not statistically significant when compared to the control animals.

The skeleton examination revealed a dose depended retardation of ossification. The main effects caused by the higher doses used (10 and 50 mg/kg) were the following: absence of one or several ossification centres of sternum, absence of the 13th rib, less than three metatarsal ossification centres, incomplete development of skull bones, distinctly larger fontanelles and wider cranial sutures. The effects were more obvious at the dose of 50 mg/kg and the signs of retardation of ossification were mainly observed at the spine. Ventral ossification centres were either completely absent, or distinctly flatter, or consisted of two separate ossif. Malformations of the spine were also observed in 4 of the 22 examined fetuses. The malformations observed were asymmetric and irregular arrangement of the main part of ossification centres. None of the mentioned malformations of ossification was observed in the control animals of this study.

Table 2. External and soft tissue observations on fetuses from dams treated orally with haloxyfop ethoxyethyl ester from day 6 to 16 of gestation[*].

Effects	Haloxyfop ethoxyethyl ester (mg/kg)			
	0	5	10	50
External observations No. of fetuses observed/No. of litters	109/10	100/10	74/10	64/10
Deformed rear appendages	0	2(2)	0	1(1)
Kinky tails	0	6(4)	5(4)	1(1)
Microphthalmia unilateral	0	0	2(2)	1(1)
Absent tail, severe edema	0	0	0	2(2)
Open nasal cavity	0	0	0	1(1)
Absent lower jaw, severe edema	0	0	0	1(1)
Severe delay of embryogenesis	0	0	0	2(1)
Cachectic fetuses	0	2(1)	5(3)	13(7)
Severe edema	0	0	0	5(4)
Soft tissue observations No. of fetuses observed/No. of litters	53/10	51/10	35/10	42/10
Ureterohydronephrosis	1(1)	6(5)	15(5)[1]	23(9)[2]

[*] :In parentheses, number of affected litters.
[1,2] : Significantly different from the control group for $P<0.01$ and $P<0.001$ respectively.

The effects observed with haloxyfop ethoxyethyl ester are in agreement with the effects reported elsewhere, related to other hypolipidemic agents. The compound AY 9944 caused vaginal haemorrhage, embryolethality and fetal malformations when tested on rats at doses 5 to 50 mg/kg. The fetuses were edematous and the most frequently observed effects were ureterohydronephrosis and holoprosencephaly (Carpent and Desclin 1968; Roux and Aubry 1966). Similar effects were observed when triparanol was tested at doses 50 to 100 mg/kg (Roux 1964; Roux et al. 1973). Embryolethality and teratogenicity were also observed when the experimental compounds M&B 30227 and M&B 31426 were tested at doses 50 and 100 mg/kg (Steele et al. 1983).

Striking differences in the teratogenic and embryolethal potential exist among hypolipidemic agents. No embryolethal or teratogenic effects were observed when MG.46 was administered to rats, rabbits and mice, at doses up to 400 mg/kg (Da Lage et al. 1972). The results from a pilot study in our laboratory with fenoxaprop-ethyl, another hypolipidemic herbicide, are indicative of a variation in the developmental toxicity of the group of phenoxy herbicides. No embryolethal or teratogenic effects were observed when fenoxaprop-ethyl was administered at a single dose of 10 mg/kg by gavage to 10 pregnant rats from the 6th to the 16th day of gestation. On the contrary slight maternal toxicity was observed in this case, as evaluated from the body weight gain.

Generally, the developmental toxicity of the hypolipidemic compounds has been associated with the blood cholesterol levels. Also a direct teratogenic action of M&B 30227 and M&B 31426 has been demonstrated from an *in vitro* study on rat embryos (Steele et al. 1983). The results from studies concerning the prevention of the developmental toxicity caused by hypolipidemic compounds, showed that the cephalic syndrome could be alleviated by a hypercholesterolemia-provoking diet while the ureterohydronephrosis was not affected (Roux 1968; Roux et al. 1979). Since haloxyfop ethoxyethyl ester did not increase the frequency of the cephalic syndrome, the developmental toxicity of the compound under the co-administration of a hypercholesterolemia-provoking diet was not studied. On the contrary, enhancement of the mainly observed effects i.e. embryo- / fetal toxicity and ureterohydronephrosis would be expected in the case of a cholesterol rich diet. A diet rich in cholesterol was found to increase significantly the fetal mortality and uro-genital malformations of rat and rabbit fetuses (Gilardy 1966; Roux et al. 1979).

Further studies are necessary to clarify the differences concerning the developmental toxicity among the group of

phenoxy herbicides and to reveal their mechanisms of action.

The above data indicate that haloxyfop ethoxyethyl ester has embryo- / fetal toxicity and teratogenic potential. The frequency of resorbed embryos and fetal malformations increased at the dose of 10 mg/kg but fetotoxicity was observed at higher doses. The most characteristic malformations, observed at a dose depended frequency, were cachectic fetuses and ureterohydronephrosis. The dose of 5 mg/kg was the lowest effective dose for the experiment. Due to the low frequency and less pronounced severity of the effects at this dose, it can be concluded that the no-observed effect level (NOEL) for the experiment is expected to be slightly lower than 5 mg/kg.

REFERENCES

Barrow VM, Taylor WJ (1969) A rapid method for detecting malformations in rat fetuses. J Morph 127, 291-306.

Carpent G, Desclin L (1968) A propos du mode d'action d'un inhibiteur de la synthèse du cholestérol, le AY 9944, sur le mécanisme gestatif et le développement foetal, chez le rat. Ann Endocrinol (Paris) 29 (4) 506-511.

Da Lage C, Labie C, Loiseau G, Lohier P, Marquet J P, Truchaud M (1972) Étude toxicologique et tératologique d'un hypocholestérolémiant (MG.46).J Eur Toxicol 5 (4) 239-253.

Gilardi T (1966) Ricerche sperimentali circa l'influenza della dieta ricca in colesterolo durante la gravidanza. Arch Ost Gin 71: 57-81.

Lalwani ND, Reddy MK, Qureshi SA, Sirtori CR, Abiko Y, Reddy JK (1983) Evaluation of selected hypolipidemic agents for the induction of peroxisomal enzymes and peroxisome proliferation in the rat liver. Human Toxicol. 2, 27-48.

Moody DE, Reddy JK (1978) Hepatic peroxisome (microbody) proliferation in rats fed plasticizers and related compounds. Toxicol Appl Pharmacol 45, 497-504.

Roux C (1964) Action tératogène du triparanol chez l'animal. Arch Fr Pediatr XXI (4) 451-464.

Roux C (1968) Essai de prévention de l'action tératogène d'un hypocholestérolémiant. C R Seances de la Soc Biol 162 (10) 1671-1675

Roux C, Aubry M (1966) Action tératogène chez le rat d'un inhibiteur de la synthèse du cholestérol, le AY 9944. C R Seances de la Soc Biol 160 (7) 1353-1357

Roux C, Aubry M, Dupuis R, Horvath C (1973) Effets tératogènes comparés du triparanol chez le Rat Wistar et le Rat Sprague-Dawley. C R Seances de la Soc Biol 167 (11) 1523-1526

Roux C, Horvath C, Dupuis R (1979) Teratogenic action and embryo lethality of AY 9944: Prevention by a hypercho

lesterolemia-provoking diet. Teratology 19: 35-38
Roux C, Taillemite JL, Aubry M, Dupuis R (1972) Effets tératogènes comparés du chlorhydrate du [trans-1,4-bis-(2-chlorobenzyl aminomethyl) cyclohexane] (AY 9944) chez le rat Wistar et le rat Sprague-Daweley. C R Seances de la Soc Biol 166 (10) 1233-1236
Steele CE, New DAT, Ashford A, Copping GP (1983) Teratogenic action of hypolipidemic agents : An *in vitro* study with postimplantation rat embryos. Teratology 28: 229-236
Stott WT (1988) Chemically induced proliferation of peroxisomes: Implication for risk assessment. Reg Toxicol Pharmacol 8, 125-129.
Wilson JG (1965) Methods for administrating agents and detecting malformations in experimental animals. In: Wilson JG, Warkany J (eds) Teratology, principles and techniques. University of Chicago Press, Chicago.

Received July 9, 1992; accepted March 12, 1993.