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Assessment of Teratological Effect and Developmental Effect of Maleic Hydrazide Salts In Rats

E. Hansen, O. Meyer, E. Kristiansen*

Institute of Toxicology, National Food Institute,
19 Mørkhøj Bygade, DK—2860 Søborg

Salts of maleic hydrazide are widely used as plant growth regulators and herbicide, (FAO 1977, IARC 1974, Khera et al, 1979). The comsumption in Denmark is approximately 22 tons per year, (DK Stat. Inf. 1981). Maleic hydrazide has been studied for mutagenicity, (Cabral and Ponomarkov 1982, FAO/WHO 1976, Speit 1983, Swietlinska and Zuk 1978) and carcinogenicity, (Cabral and Ponomarkow 1982, FAO/WHO 1976, Hejden 1981, IARC 1974). Only few toxicological studies for the assessment of possible effect on reproduktion have been performed, (FAO/WHO 1976, Khera et al 1979), and more knowledge about possible teratogenic effect is required (FAO/WHO 1976). The two compounds, (MEA-MH) monoethanolamine maleic hydrazide and (Na-MH) sodium-maleic hydrazide (Na-MH) have been subjected to an investigation in rats for teratological effect, and the latter for assessment of developmental effect of an exposure during gestation and lactation as well.

MATERIAL AND METHODS

The compounds, the monoethaneolamine and the sodium salt of maleic hydrazide were obtained from manufacturer (Kemisk Værk Køge, Lyngvej 2, DK-4600 Køge, Denmark). The MEA-MH was an aqueos solution, 360g/l, PH 8-8,5, hydrazine less than 15 ppm. Na-MH was a powder, 97% w/w Na-MH, 73% w/w maleic hydrazide, hydrazine 46 ppm.

The rats were outbred Mol: WIST (Møllegaards Breeding Centre Ltd., Tornbjergvej 40, Ejby, DK-4623, Ll. Skensved), 9 weeks of age at arrival and 11 weeks at commencement of the experiments. The rats were kept in stainless steelwire cages at 23 $\pm$ 1°C, rel. humidity 60 $\pm$ 5%, 6-88 air changes/h and electric light from 7 pm to 7 am.

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* Present adress: National Institute of Occupational Health, 73 Baunegaardsvej, DK - 2900, Hellerup.

Food: Ground commercial chow (exp. IB and IIB: Rat diet, Møllegaards Breeding Centre, exp. IA, IIA and III: Brood Stock Feed for Rats & Mice-R3/, Astra-Ewos AB, Box 618, S-15127 Södertälje, Sweden) and water (acidified, citric acid to PH 3.5 for preservation) were available ad libitum.

Table I. Design, exp. I and exp. II.

Group	MEA-MH No. of rats	Dose mg/kg b.w.	Dosing period (days)	NA-MH No. of rats	Dose mg/kg b.w.	Dosing period (days)
		Exp.IA			Exp.IIA	
I	24	b	6-15	23	a	6-15
II	24	500	6-15	27	500	6-15
III	24	1500	6-15	24	1500	6-15
IV	22	3000	6-15	28	3000	6-15
V	12	3000	6- 9	12	3000	6- 9
VI	13	3000	9-12	12	3000	9-12
VII	12	3000	12-15	12	3000	12-15
VIII	26	3000	1-21	24	3000	1-21
		Exp.IB			Exp.IIB	
I	53c	b	6-15	53c	b	6-15
II	44	500	6-15	44	500	6-15
III	44	1500	6-15	44	1500	6-15
IV	44	3000	6-15	40	3000	6-15
V	44	3000	1-21	40	3000	1-21

a) Except group VIII all groups dosed by gavage. b) 10 ml H_2O/kg b.w. by gavage. c) Common control group for experiments IB and IIB.

The rats in exp. IA and IIA were mated, 1 female per male per cage when 11 weeks old. The presence of plug, (Kalman et al, 1969) was checked every evening (designated day 0) and morning (designated day 1). Mated females were transferred singly to plastic cages and weighed day 1, 6, 15 and 21. The males were omitted from study after completed mating.

The MEA-MH and Na-MH were dissolved in H_2O and dosed to rats by gavage 10 ml per kg b.w. according to table 1. Group VIII, however, received the compounds mixed in the diet (concentration based upon a food consumption of 70 g/kg b.w./day).

On day 21 the rats were weighed, sacrified by exsanguination under CO_2 anasthesia and necropsied. The following data were recorded: Weight of the intact uteri and ovaries, number of corpora luteae (CL), of implantations and fetuses, alive and dead. The fetuses were weighed, sexed and examined for gross

external malformations. Half of the fetuses were examined for skeletal anomalies, (Dawson 1926). The remaining fetuses were fixed in Bouins solution and sectioned according to the method of Wilson, (1965) in order to detect internal malformations of the soft tissues. Weight gain of the dams, sex ratio of fetuses and pre- and postimplantation loss, were calculated (Meyer and Hansen, 1975).

Experiments IB and IIB are repetitions of the experiments IA and IIA. In order to ensure the intake of the total dose, all groups were dosed by gavage (table 1). In addition food consumption was measured.

Mating procedure in exp. III was as described above. The mated females were placed by twos in plastic cages until 3 days before expected delivery, when they were housed separately. The dams received MEA-MH mixed in the diet from day 6 of gestation to day 21 post partum. Body weight was recorded day 1, 6, 15 and 21. Dates of the birth of F_1, rats were recorded as well as litter size and sex ratio. The duration of the gestation period was estimated. Twenty-four hours after birth the litter size was reduced to 8, and equal sex distribution was achieved. Weight of the litter was recorded before and after standardization, and half the litter was crossfostered to obtain the following groups:

Design, exp. III

Dose of MEA-MH	Number of Litters	F_0/F_1	Number of Litters
I	47	I/I	25
0 mg		I/II	20
II	45	II/II	25
3000 mg		II/I	20

The F_1 animals were weighed on days 1, 7, 14, and 21. The days of appearance of pinna unfolding, tooth eruption, startle response to an auditory stimulus and opening of the eyes were recorded, (Meyer and Hansen, 1980). The development of neuromuscular function and orientation responses was assessed by static righting reflex, cliff avoidance, righting reflex, olfactory orientation, visual orientation (choice of jumping"), rotarod, (Meyer and Hansen, 1980) and ability to cross a narrow path (two glass rods, 3 mm in diameter, 1 cm in between and 60 cm long), (Barlow et al, 1978).

Student's t-test was performed on body weight F_0, weight gain F_0, litter size, sex ratio, body weight F_1, mean weight of the intact uteri (incl. ovaries), pre- and postimplantation loss.

The Quick Test-chi-square was used for special parameters in the teratogenicity tests (Mainland, 1963).

Kruskal-Wallis one-way analysis of variance was used for developmental parameters, (Siegels 1956).

RESULTS AND DISCUSSION

In the first experiment the rats dosed with 3000 mg MEA-MH/kg b.w. in the diet (group VIII) exibited a significant decrease in body weight and weight gain. This effect was not observed in the repeated study, dosing the rats by gavage and no significant effect on food consumption was observed.

Reproduction data for group VIII, experiment IA, demonstrated a marked effect of 3000 mg MEA-MH/kg b.w., when given in the diet. Thus the number of resorptions was higher and the mean weight of the fetuses was lower compared to the control ($p < 0.001$). In the corresponding group, group V in experiment IB a similar but less pronounced effect was observed ($p < 0,5$ and $p < 0,001$ resp.). In addition the post implantation loss was increased significant ($p < 0,01$) in both experiments.

In table II, the incidences of malformation are listed. The figures for major malformations in the first experiment show a somewhat higher incidence in the dosed groups in comparison to the control group. This was not observed in the repeated study. Delayed ossification of sternebrae ($p < 0.001$) and in other bones apart from the skull ($p < 0.05$) was seen in group VIII. No other effect was observed in any of the parameters.

During the dosing periode none of the dams showed any signs of effect of Na-MH apart from a reduction in food consumption day 1-6 in group V, experiment IIB. The reproduction data did not reveal any effect of the dosing.

The incidences of malformations are listed in table II. As for experiment IA, (MEA-MH) a somewhat higher incidence of major visceral malformations was seen in the first Na-MH-experiment, IIA. The figures for minor skeletal malformations/variations show an increased incidence in groups III and V in experiment IIB. No other effect was observed in any of the parameters.

Weight gain was significantly reduced in the dosed animals in exp. III from the initation of the MEA-MH dosing, day 6 up to day 15 in the gestation period ($p < 0.001$). In the same group, the body weight was slightly lower, than that of the controls day 15 and day 21 ante partum ($p < 0.05$).

The reproduction data did not reveal any effect of MEA-MH in respect to pregnancy rate, duration of pregnancy and litter

size. The average birth weight in the dosed group was 5,9 g for the males and 5,6 g for the females compared to 6,3 g and 6,0 g for males and females respectively in the control group ($p < 0.01$ for the males and $p < 0.001$ for the females).

A significant effect upon the body weight up to age 35 days of F_1 animal nursed by dosed mothers was demonstrated (groups II/II and II/I). The data on developmental parameters showed a significant delay in startle response to an auditory stimulus day 13 for both males and females nursed by dosed females (males II/II and II/I, $p < 0.001$; females II/II and II/I $p < 0.001$ and $p < 0.01$ resp.).

Apart from a delay in pinna unfolding day 3 in pups dosed during gestation (groups I/II and II/II) no consistent effect of MEA-MH was observed in any of the other developmental parameters.

Average relative brain weight was signicantly higher (males: I/II: $p < 0.01$, II/II AND II/I: $p < 0.001$ and females: I/II $p < 0.05$, II/II: $p < 0.001$, II/I: $p < 0.01$) in animals of both sexes in all dosed groups compared to the controls. This effect was most pronounced in animals in groups II/II and II/I.

The F_0 dams, experiment IA and experiment III, fed 3000 mg MEA-MH per kg b.w. exhibited a significant decrease in body weight and weight gain. This toxic effect is in correspondance with that reported from studies feeding rats with the diethanolamine salt (FAO/WHO 1976). The same dose given to rats by gavage (experiment IB) did not result in a similar growth depression, and no alterations in food consumption was demonstrated. The effect of MEA-MH when fed to rats could be explained by an alterration in the palatability of the diet resulting in a decrease in food consumption.

In 3000 mg groups MEA-MH demonstrated a significant effect on reproduction while this was not the case with Na-MH. This result supports earlier findings in experiments with the diethanolamine salt and the sodium salt (FAO/WHO 1976). The effect of MEA-MH could be due to the monoethanolamine moiety as indicated for the related diethanolamine (FAO/WHO 1976). However, no data on the dissociation of the MEA-MH in the gastrointestinal tract in rats are available.

The results from experiments I and II, A and B do not indicate an evident teratological effect of any of the substances. MEA-MH and Na-MH are only very weak teratogens at doses higher than 500 mg/b.w. if teratogenic at all. This is in accordance with previous experiments with maleic hydrazide (Khera et al, 1979).

Table 2. Incidence of malformation.

IIa No. of litters (pups) affected. (Letters refer to diagnosis table 3).

	Exp. A								Exp. B				
Group	I	II	III	IV	V	VI	VII	VIII	I	II	III	IV	V
MEA-MH													
Total no. examined	21(224)	21(229)	22(231)	21(201)	12(151)	12(136)	11(134)	19(153)	47(543)	42(477)	41(466)	41(466)	38(420)
Gross malformation	0	0	2(2)[b,k]	0	0	0	1(1)[n]	1(1)[b]	1(1)[i,k]	1(1)[l]	1(1)[m]	0	2(2)[d,m]
Skeletal examination													
Total no.	21(111)	21(116)	22(115)	21(101)	12(74)	12(68)	11(68)	19(77)	47(273)	42(241)	40(231)	41(232)	38(208)
Minor variation[a]	13(17)	7(9)	10(12)	11(26)	6(6)	6(10)	2(6)	16(27)	23(39)	28(49)	32(60)**	29(43)*	24(33)
Major defects	0	0	1(1)[b]	0	0	0	0	1(2)[c]	0	0	0	0	1(1)[d]
Visceral examination													
Total no.	20(113)	21(113)	21(116)	21(100)	12(77)	12(68)	11(66)	19(76)	47(270)	42(236)	41(235)	41(234)	38(212)
Major defects	0	0	1(1)[cc]	1(1)[o]	1(1)[v]	0	1(1)[x]	1(1)[o]	2(2)[g,m,y]	1(1)[z]	2(2)[z,oe]	0	1(2)[z,m]
IIb													
Na-MH													
Total no examined	20(211)	21(231)	21(214)	21(231)	10(115)	10(119)	11(115)	23(260)	47(543)	42(451)	42(496)	33(354)	34(386)
Gross malformation	0	0	1(1)[e]	1(1)[f]	0	1(1)[g]	0	0	1(1)[i,k]	0	2(2)[g,f;l]	1(1)[h]	0
Skeletal examination													
Total no.	20(107)	21(114)	21(104)	21(117)	10(59)	10(59)	11(57)	23(130)	47(273)	41(229)	42(248)	33(178)	34(191)
Minor variation[a]	9(9)	9(9)	6(6)	3(4)	3(5)	7(10)	5(7)	11(15)	23(39)	27(41)	30(59)*	21(31)	24(53)*
Major defects	0	0	0	0	0	0	0	0	0	0	0	0	0
Visceral examination													
Total no.	19(104)	21(117)	21(110)	21(114)	10(56)	10(60)	11(58)	23(130)	47(270)	42(222)	42(248)	33(176)	33(195)
Major defects	1(1)[o]	0	1(1)[e]	3(3)[v,f,p]	0	1(1)[q]	1(1)[r]	2(2)[m,p]	2(2)[g,m,y]	0	1(1)[g]	1(1)[s]	2(2)[t,u]

* $p < 0,1$ ** $p < 0,01$

Table 3. Diagnosis codes

a	Asymmetrical and bipartite sternebrae, wavy ribs, fused ribs, single bipartite or other variations in thoracic vertebrae, rudiment of cervical rib.
b	Cranioschisis
c	Severely underdeveloped
cc	Heart defect, microcephalia, anophthalmia
d	Micromelia
e	Atresia ani
f	Eventratio abdominalis
g	Hernia umbilicalis
h	Prognathia
i	Spina bifida
k	Exencephali
l	Still born
m	Hydrocephalus
n	Acauda
o	Anophthalmia uni- or bilateralis
p	Hernia diaphragmatica
q	Situs inversus
r	Haemopericardium
s	Aplasia of the right testicle
t	Haemophthalmia
u	Microphthalmia
v	Haemocoelia
x	Malformed kidneys, ovarian aplasia
y	Unilateral kidney dysplasia
z	Unilateral hydronephrosis
oe	Cleft palate

The results from experiment III show a significant depression on body weight and weight gain of rats nursed by MEA-MH dosed mothers. The effect on body weight persist, while the weight gain is comparable among the groups after weaning. Whether this lactation effect is a consequence of a toxic effect on the mother, a direct effect of MEA-MH on the pups via the milk or both cannot be established from this study. A reduced weaning weight has previously been demonstrated in an experiment with sodium salt of maleic hydrazide (Oser, 1955). The increase in relative brain weight is considered a result of the lower body weight.

The delay in startle response to an auditory stimulus in rats nursed by MEA-MH dosed mothers is considered a significant effect as it is observed in both sexes. This observed delay cannot be explained.

In conclusion the Na-MH in the above experiments appears to be less toxic than MEA-MH. For both substances no indication of toxicological effect was demonstrated in the lowest dose level in any of the teratological studies, which is at least hundred-fold higher than those relevant for human exposure (FAO, 1977). A no-effect level from the results of the study for assessment of developmental effect only using one dose could not be established.

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