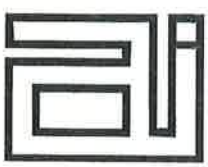
Adverse effects of Ethyleneglycol on fertility and reproduction

A literature study

Conducted at the request of the Directorate General of Labour by:
the TNO-CIVO Toxicology and Nutrition Institute;
in cooperation with:
the Coronel Laboratory of Occupational and Environmental Health,
Faculty of Medicine, University of Amsterdam

Directoraat-Generaal van de Arbeid



S 73-9

Adverse effects of Ethyleneglycol on fertility and reproduction

A literature study

Conducted at the request of the Directorate General of Labour by:
the TNO-CIVO Toxicology and Nutrition Institute;
in cooperation with:
the Coronel Laboratory of Occupational and Environmental Health,
Faculty of Medicine, University of Amsterdam

Authors:
Herman B. W. M. Koëter
Willem G. H. Blijleven
Henriëtte C. Dreef-van der Meulen (TNO-CIVO)

Anne Stijkel
Reinier L. Zielhuis (Coronel Laboratory)

Supervision on behalf of the initiator:
G. Idema

October 1989

ISBN 90 5307 013 3

CONTENTS Page

ADVERSE EFFECTS OF ETHYLENEGLYCOL ON FERTILITY AND REPRODUCTION

1. Introduction

Although an extensive literature survey was conducted with respect to human data, no such data were found. Therefore, the evaluation was only based on animal data.

2. Animal data

2.1 Risks of exposure of female animals with respect to gonads, endocrine system and fertility

No data available.

2.2 Risks of exposure of female animals with respect to gestation and prenatal development

Maronpot et al. (1983) administered ethylene glycol in the diet to rats (N=20 per group) on days 6 through 15 of gestation. The doses of ethylene glycol administered were 0, 40, 200 or 1000 mg/kg/day. Each dam was killed on day 21 of gestation. No significant differences in maternal body weight gain were observed between control and treated animals on day 6, 11, or 21. Treatment with ethylene glycol did not significantly alter fetal length, fetal body weight, total number of implantations, or litter size. In the 1000 mg/kg/day group, pre-implantation loss (23%) was higher than in the control group (10%), but this difference was not statistically significant. The incidence of major malformations in litters of ethylene glycol treated females was not increased. Statistically significant increased incidences of poorly ossified and unossified vertebral centra in fetuses of the 1000 mg/kg/day group was observed. Price at al. (1985) administered rats (N= 28 or 29) ethylene glycol daily by gavage with 0, 1250, 2500 or 5000 mg/kg/day on days 6 through 15 of gestation. On day 20 the dams were killed. The study was designed to elucidate the structural developmental effects (consisting of cleft lip, fused ribs, abnormally shaped vertebrae and sternebrae) observed by Lamb et al. (1985) in rats (see 2.6), and the reduced growth and viability of offspring reported by Hazelden (1983) (see 2.3).

Maternal weight gain was statistically (P<0.05) decreased in the mid- and high dose group. In the paper are no data concerning food consumption. Maternal water consumption was significantly (P<0.05) increased in the mid- and high dose group. Treatment related changes in organ weights consisted of decreased absolute liver weights (P<0.05), but not of the relative weights in the high dose group and increased relative kidney weights in the mid- and high dose group (P< 0.01). Absolute kidney weights were not affected. In the high dose group post-implantation loss was significantly increased (P<0.05). In the mid- and high dose groups the number of live fetuses per litter was reduced (P<0.05). In addition, in both these groups when compared with the controls the average fetal body weight was decreased (P<0.01), and the percentage of malformed fetuses per litter was increased (P<0.01). The number of litters with one or more malformed fetuses was increased (P<0.001) in all dose groups. The external malformations most regularly observed consisted of cleft palate, cleft lip, anophthalmia, meningoencephalocele, and occurred in a significantly higher number of litters in the high-dose group than in controls. Fetuses with visceral malformations (anomalies of the great vessels, hydroureter, hydronephrosis) occurred in all groups. Fetuses with skeletal malformations (fused ribs, fused sternebrae) occurred in a significantly (P<0.001) higher number of litters in the mid- and high dose groups than in the control group.
Price et al. (1985) also exposed mice (N= 25, 26, or 27) to ethylene glycol at dose levels of 0, 750, 1500 or 3000 mg/kg/day by gavage on days 6 through 15 of gestation. On day 17 the dams were killed. Maternal body weight at termination (day 17), maternal weight gain during the treatment period and during gestation, gravid uterine weight and absolute maternal liver weight (g) were significantly (P<0.01) decreased in the mid and high dose groups. A significant reduction in the number of implantation sites per litter was seen in the mid dose group, but not in the high dose group, and was therefore not considered to be treatment related. The average fetal body weight per litter was statistically (P<0.01) decreased in all dose groups. The number of live fetuses per litter was significantly (P<0.05) reduced in the high dose group. In all treated groups the percentage of malformed fetuses per litter (P<0.01) and the percentage of litters with one or more malformed fetuses were significantly (respectively P<0.01 and P<0.001) increased in comparison to the controls. External malformations (exencephaly, cleft palate, cleft lip, facial cleft), or visceral malformations (hydroureter, hydronephrosis) occurred in a high number of litters in the high-dose group.

Fetuses with skeletal malformations (fusion or malformation of the ribs, vertebral arches, centra, and sternebrae) occurred in a higher number of litters in all groups.
In the dominant lethal study of DePass et al. (1986), 15 F2 rats per dosage group (0, 0.04, 0.2 and 1.0 g/kg/day) from a three-generation reproduction study (see B2) were removed from their ethylene glycol regiment (both F0 and F1 males and females had ethylene glycol in their diet) at 155 days of age and bred to three seperate sets (one set per week) of 15 untreated females. Each female was killed on day 12 of gestation and the ovaries and uteri were examined for the number of live and dead fetuses. Ethylene glycol administration did not lead to statistically significant adverse effects on the number of females with implants, total number of implants, number of dead implants, number of live implants and the dominant lethal mutation frequency measured in the three mating intervals.

2.3 Risks of exposure of female animals with respect to the offspring

Hazelden (1983) exposed mice (N=50) to 11.16 g ethylene glycol/kg/day po on gestational days 8 through 15. This exposure was toxic to the dams (5/50 = 10% mortality). Within 12 hours after delivery, the number of live and stillborn pups was recorded. The ethylene glycol treated mice showed a significant reduction ($P<0.05$) in viable litters (41%). Postnatal observations showed, that ethylene glycol reduced the number of live pups per litter ($P<0.05$), increased the number of death pups per litter ($P<0.05$), reduced pup survival ($P<0.05$), reduced pup birth weight ($P<0.05$), and reduced pup weight gain over days 1 to 3 postpartum ($P<0.05$). (literature reference: the papers of Maronpot et al. 1983; Price et al. 1985; Schuler et al. 1984).

2.4 Risks of exposure of male animals with respect to gonads, endocrine system and fertility

Nagano et al. (1984) administered ethylene glycol to male mice (N=5 per group) in doses of 0, 500, 1000, 2000 or 4000 mg/kg bodyweight 5 days per week for 5 weeks by gavage. One day after the last day of administration, animals were necropsied. No significant difference in weight of the testis was found in the ethylene glycol group when compared with control animals. Histopathological examination of the testis did not reveal any dose-related atrophy of the

seminiferous epithelium. In addition Sertoli cells and Leydig cells did not reveal abnormalities.

2.5 Risks of exposure of male animals with respect to gestation of the partner and the offspring

No data available.

2.6 Risks of exposure of both male and female animals (mating partners) with respect to gestation and the offspring

Lamb et al. (1985) administered 0, 0.25, 0.5 or 1% ethylene glycol (respectively approximately 0, 0.4, 0.8 or 1.6 g/kg/day) in drinking water. Each dose group consisted of 20 males and 20 females. The mice were exposed during a 7-day premating period, after which they were randomly paired (one male to one female) within each dose group and cohabited for 98 days while treatment continued. Newborn litters were evaluated and immediately killed. At the end of the 98-day cohabitation period males and females were separated to prevent further mating; litters of the 0 and 1% dose group were kept during the following 21-day segregation period. Ethylene glycol exposure (0 or 1% in the drinking water) was continuous during the 98-day cohabitation period and the 21-day segregation period which followed, and throughout the life of the offspring born during the 21 days. The litters born during the 21-day segregation period were weaned and evaluated for reproductive performance. No treatment-related effects were observed in the mating partners with respect to body weight, water consumption or adverse clinical signs. Exposure to 1% ethylene glycole in the drinking water was associated with statistically significant decreases in the average number of litters per mating pair ($P<0.01$), the mean average number of live pups per litter ($P<0.05$) and the mean average live pup weight ($P<0.05$).
During the F1-generation (consisting of 20 males and 20 females per dose group) exposure to the test substance was continued and the animals were mated when approximaly 70 days of age. In the F1 generation the fertility (no. pregnant/no. mated) was 80% in the control group compared with 61% in the group receiving 1% ethylene glycol, but the difference was not statistically significant. The number of live pups per litter and the live pup weight were

slightly lower in the ethylene glycol treated group (control 1.65 ± 0.03g, treated 1.62 ± 0.07g), but the differences were not statistically significant. Although no clinical signs of ethylene glycol toxicity were observed, different facial features were noticed in some of the offspring of the treated mice but not in the controls. The affected mice had a shorter snout with wide-set eyes. Necropsy of the F1 offspring revealed a pattern of skeletal defects in the treated mice affecting the skull, sternebrae, ribs and vertebrae in both male and females. The various defects were mentioned in the paper, but the data were not quantitatively presented.

To assess the possible effects of ethylene glycol on reproductive performance and mutagenesis, three-generation reproduction and dominant lethal studies (result: see F2) were performed in rats by DePass et al. (1986). Ethylene glucol was included in the diet at dosages of 0, 0.04, 0.2 and 1.0 g/kg/day during three generations of reproduction. Administration of ethylene glycol to the F0 rats began at 7 weeks of age. At 100 days of age 10 males were mated to 20 females (1 male was housed with 2 females). The date of parturition and the number of live and dead newborn were recorded for each litter. The appearance and behavior of dams and pups were observed daily. Offspring were weighed as litters at 4 and 14 days and individually at 21 days postpartum, the day they were weaned. F1 and F2 rats were randomly selected within each dosage group and treated as described for the F0 rats. Brother and sister mating were avoided for each generation. Necrospsies were performed on five males and five females randomly selected from each dosage level of the F2 parents and the F3 weanlings. Throughout the study there was no effect of ethylene glycol treatment on body weight gain or diet consumption, nor was there any mortality among parental rats. No treatment-related effect for the reproductive indices was observed for all three generations. The reproductive indices were fertility index, gestation (survival) index, 0-4, 4-14 and 4-21 survival index, and days from first mating to litter. There were no treatment-related histopathologic findings in the F2 parents or in the F3 weanlings.

The difference between the results of Lamb et al. (1985) and DePass et al. (1986) may be explained by differences in species sensitivity, route of administration, and dose of ethylene glycol. The importance of the species difference was suggested by the results of the Price et al. (1985) (see 2.2) study in which mice were much more sensitive than rats to the teratogenic effect of ethylene glycol.

2.7 Conclusion animal data

With respect to maternal toxicity it can be concluded that in the two rat studies the NAEL was established at 1000 mg/kg/day [Maronpot (1983), Price (1985)], and in the mouse study on 750 mg/kg/day [Price (1985)]. Sufficient data is lacking to draw a well documented conclusion about the risks of exposure of female animals with respect to the gonads, endocrine system and fertility (10.2.2). Concerning the risks of exposure of female animals with respect to gestation and prenatal development it can be concluded that ethylene glycol is embryotoxic when administered orally, inducing postimplantation loss and reduced fetal body weight. In addition, ethylene glycol appeared to be teratogenic in the rat and mouse. The types of malformations observed included cleft lip, fused ribs, abnormally shaped vertebrae, shortened frontal nasal and parietal bones [Price et al. (1985), Lamb et all. (1985)]. For ethylene glycol the NAEL might be lower than 750 mg/kg/day (oral administration).
Data are lacking to draw a well documented conclusion about the risks of exposure of male animals with respect to gonads, endocrine system, fertility, gestation of the partner and risk to the offspring.
The present MAC (125 mg/m^3) is not far below the dose levels that caused malformations after oral administration. Therefore, more research is needed to establish a well documented NAEL.

3. Literature

DePass, L.R., M.D. Woodside, R.R. Maronpot and C.S. Weil (1986)
Three-generation reproduction and dominant lethal mutagenesis studies of ethylene glycol in the rat.
Fundamental and Applied Toxicology 7:566-572

Lamb IV, J.C., R.R. Maronpot, D.K. Gulati, V.S. Russell, L. Hommel-Barnes and P.S. Sabharwal (1985)
Reproductive and developmental toxicity of ethylene glycol in the mouse.
Toxicology and Applied Pharmacology 81:100-112

Maronpot, R.R., J.P. Zelenak, E.V. Weaver and N.J. Smith (1983)
Teratogenicity study of ethylene glycol in rats.
Drug and chemical toxicology 6 (6):579-594

Nagano, K., E. Nakayama, H. Oobayashi, T. Nishizawa, H. Okuda and K. Yamazaki (1984)
Environmental Health Perspectives 57:75-84

Price, C.J., C.A. Kimmel, R.W. Tyl and M.C. Marr (1985)
The development toxicity of ethylene glycol in rats and mice.
Toxicology and applied pharmacology 81:113-127

Schuler, R.L., D.D. Hardin, R.W. Niemeier, G. Booth, K. Hazelden, V. Piccirillo and K. Smith (1984)
Results of testing fifteen glycol ethers in a short-term in vivo reproductive toxicity assay.
Environmental Health Perspectives 57:141-146