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Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M Street., S.W.
Washington, D.C. 20460
Attn: Section 8(e) Coordinator (CAP Agreement)

Dear Coordinator:

8ECAP-0025

On behalf of the Regulatee and pursuant to Unit II B.1.b. and Unit II C of the 6/28/91 CAP Agreement, E.I. Du Pont de Nemours and Co. hereby submits (*in triplicate*) the attached studies. Submission of this information is voluntary and is occasioned by unilateral changes in EPA's standard as to what EPA now considers as reportable information. Regulatee's submission of information is made solely in response to the new EPA §8(e) reporting standards and is not an admission: (1) of TSCA violation or liability; (2) that Regulatee's activities with the study compounds reasonably support a conclusion of substantial health or environmental risk or (3) that the studies themselves reasonably support a conclusion of substantial health or environmental risk.

The "Reporting Guide" creates new TSCA 8(e) reporting criteria which were not previously announced by EPA in its 1978 Statement of Interpretation and Enforcement Policy, 43 Fed Reg 11110 (March 16, 1978). The "Reporting Guide states criteria which expands upon and conflicts with the 1978 Statement of Interpretation. Absent amendment of the Statement of Interpretation, the informal issuance of the "Reporting Guide" raises significant due processes issues and clouds the appropriate reporting standard by which regulated persons can assure TSCA Section 8(e) compliance.

For Regulatee,

Mark H. Christman
Counsel
Legal D-7158
1007 Market Street
Wilmington, DE 19898
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INIT 10/27/92



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ATTACHMENT 1

Submission of information is made under the 6/28/91 CAP Agreement, Unit II. This submission is made voluntarily and is occasioned by recent changes in EPA's TSCA §8(e) reporting standard; such changes made, for the first time in 1991 and 1992 without prior notice and in violation of Regulatee's constitutional due process rights. Regulatee's submission of information under this changed standard is not a waiver of its due process rights; an admission of TSCA violation or liability, or an admission that Regulatee's activities with the study compounds reasonably support a conclusion of substantial risk to health or to the environment. Regulatee has historically relied in good faith upon the 1978 Statement of Interpretation and Enforcement Policy criteria for determining whether study information is reportable under TSCA §8(e), 43 Fed Reg 11110 (March 16, 1978). EPA has not, to date, amended this Statement of Interpretation.

After CAP registration, EPA provided the Regulatee the June 1, 1991 "TSCA Section 8(e) Reporting Guide". This "Guide" has been further amended by EPA, EPA letter, April 10, 1992. EPA has not indicated that the "Reporting Guide" or the April 1992 amendment supersedes the 1978 Statement of Interpretation. The "Reporting Guide" and April 1992 amendment substantively lowers the Statement of Interpretation's TSCA §8(e) reporting standard². This is particularly troublesome as the "Reporting Guide" states criteria, applied retroactively, which expands upon and conflicts with the Statement of Interpretation.³ Absent amendment of the Statement of Interpretation, the informal issuance of the "Reporting Guide" and the April 1992 amendment clouds the appropriate standard by which regulated persons must assess information for purposes of TSCA §8(e).

²In sharp contrast to the Agency's 1977 and 1978 actions to soliciting public comment on the proposed and final §8(e) Policy, EPA has unilaterally pronounced §8(e) substantive reporting criteria in the 1991 Section 8(e) Guide without public notice and comment. See 42 Fed Reg 45362 (9/9/77), "Notification of Substantial Risk under Section 8(e): Proposed Guidance".

³A comparison of the 1978 Statement of Interpretation and the 1992 "Reporting Guide" is appended.

Throughout the CAP, EPA has mischaracterized the 1991 guidance as reflecting "longstanding" EPA policy concerning the standards by which toxicity information should be reviewed for purposes of §8(e) compliance. Regulatee recognizes that experience with the 1978 Statement of Interpretation may cause a review of its criteria. Regulatee supports and has no objection to the Agency's amending reporting criteria *provided that* such amendment is not applied to the regulated community in an unfair way. However, with the unilateral announcement of the CAP under the auspices of an OCM enforcement proceeding, EPA has wrought a terrific unfairness since much of the criteria EPA has espoused in the June 1991 Reporting Guide and in the Agency's April 2, 1992 amendment is new criteria which does not exist in the 1978 Statement of Interpretation and Enforcement Policy.

The following examples of new criteria contained in the "Reporting Guide" that is not contained in the Statement of Interpretation follow:

- o even though EPA expressly disclaims each "status report" as being preliminary evaluations that should not be regarded as final EPA policy or intent⁴, the "Reporting Guide" gives the "status reports" great weight as "sound and adequate basis" from which to determine mandatory reporting obligations. ("Guide" at page 20).
- o the "Reporting Guide" contains a matrix that establishes new numerical reporting "cutoff" concentrations for acute lethality information ("Guide" at p. 31). Neither this matrix nor the cutoff values therein are contained in the Statement of Interpretation. The regulated community was not made aware of these cutoff values prior to issuance of the "Reporting Guide" in June, 1991.
- o the "Reporting Guide" states new specific definitional criteria with which the Agency, for the first time, defines as 'distinguishable neurotoxicological effects': such criteria/guidance not expressed in the 1978 Statement of Interpretation.⁵
- o the "Reporting Guide" provides new review/ reporting criteria for irritation and sensitization studies; such criteria not previously found in the 1978 Statement of Interpretation/Enforcement Policy.
- o the "Reporting Guide" publicizes certain EPA Q/A criteria issued to the Monsanto Co. in 1989 which are not in the Statement of Interpretation; have never been published in the Federal Register or distributed by the EPA to the Regulatee. Such Q/A establishes new reporting criteria not previously found in the 1978 Statement of Interpretation/Enforcement Policy.

⁴The 'status reports' address the significance, if any, of particular information reported to the Agency, rather than stating EPA's interpretation of §8(e) reporting criteria. In the infrequent instances in which the status reports contain discussion of reportability, the analysis is invariably quite limited, without substantial supporting scientific or legal rationale.

⁵ See, e.g., 10/2/91 letter from Du Pont to EPA regarding the definition of 'serious and prolonged effects' as this term may relate to transient anesthetic effects observed at lethal levels; 10/1/91 letter from the American Petroleum Institute to EPA regarding clarification of the Reporting Guide criteria.

In discharging its responsibilities, an administrative agency must give the regulated community fair and adequate warning to as what constitutes noncompliance for which penalties may be assessed.

Among the myriad applications of the due process clause is the fundamental principle that statutes and regulations which purport to govern conduct must give an adequate warning of what they command or forbid.... Even a regulation which governs purely economic or commercial activities, if its violation can engender penalties, must be so framed as to provide a constitutionally adequate warning to those whose activities are governed.

Diebold, Inc. v. Marshall, 585 F.2d 1327, 1335-36 (D.C. Cir. 1978). See also, Rollins Environmental Services (NJ) Inc. v. U.S. Environmental Protection Agency, 937 F. 2d 649 (D.C. Cir. 1991).

While neither the are rules, This principle has been applied to hold that agency 'clarification', such as the Statement of Interpretation, the "Reporting Guide" nor the April 1992 amendments will not applied retroactively.

...a federal court will not retroactively apply an unforeseeable interpretation of an administrative regulation to the detriment of a regulated party on the theory that the post hoc interpretation asserted by the Agency is generally consistent with the policies underlying the Agency's regulatory program, when the semantic meaning of the regulations, as previously drafted and construed by the appropriate agency, does not support the interpretation which that agency urges upon the court.

Standard Oil Co. v. Federal Energy Administration, 453 F. Supp. 203, 240 (N.D. Ohio 1978), aff'd sub nom. Standard Oil Co. v. Department of Energy, 596 F.2d 1029 (Em. App. 1978):

The 1978 Statement of Interpretation does not provide adequate notice of, and indeed conflicts with, the Agency's current position at §8(e) requires reporting of all 'positive' toxicological findings without regard to an assessment of their relevance to human health. In accordance with the statute, EPA's 1978 Statement of Interpretation requires the regulated community to use scientific judgment to evaluate the significance of toxicological findings and to determining whether they reasonably support a conclusion of a substantial risk. Part V of the Statement of Interpretation urges persons to consider "the fact or probability" of an effect's occurrence. Similarly, the 1978 Statement of Interpretation stresses that an animal study is reportable only when "it contains reliable evidence ascribing the effect to the chemical." 43 Fed Reg. at 11112. Moreover, EPA's Statement of Interpretation defines the substantiality of risk as a function of both the seriousness of the effect and the probability of its occurrence. 43 Fed Reg 11110 (1978). Earlier Agency interpretation also emphasized the "substantial" nature of a §8(e) determination. See 42 Fed Reg 45362, 45363

(1977). [Section 8(e) findings require "extraordinary exposure to a chemical substance...which critically imperil human health or the environment"].

The recently issued "Reporting Guide" and April 1992 Amendment guidance requires reporting beyond and inconsistent with that required by the Statement of Interpretation. Given the statute and the Statement of Interpretation's explicit focus on substantial human or environmental risk, whether a substance poses a "substantial risk" of injury requires the application of scientific judgment to the available data on a case-by-case basis.

If an overall weight-of-evidence analysis indicates that this classification is unwarranted, reporting should be unnecessary under §8(e) because the available data will not "reasonably support the conclusion" that the chemical presents a substantial risk of serious adverse consequences to human health.

Neither the legislative history of §8(e) nor the plain meaning of the statute support EPA's recent lowering of the reporting threshold that TSCA §8(e) was intended to be a sweeping information gathering mechanism. In introducing the new version of the toxic substances legislation, Representative Eckhart included for the record discussion of the specific changes from the version of H. R. 10318 reported by the Consumer Protection and Finance Subcommittee in December 1975. One of these changes was to modify the standard for reporting under §8(e). The standard in the House version was changed from "causes or contributes to an unreasonable risk" to "causes or significantly contributes to a substantial risk". This particular change was one of several made in TSCA §8 to avoid placing an undue burden on the regulated community. The final changes to focus the scope of Section 8(e) were made in the version reported by the Conference Committee.

The word "substantial" means "considerable in importance, value, degree, amount or extent". Therefore, as generally understood, a "substantial risk" is one which will affect a considerable number of people or portion of the environment will cause serious injury and is based on reasonably sound scientific analysis or data. Support for the interpretation can be found in a similar provision in the Consumer Product Safety Act. Section 15 of the CPSA defines a "substantial product hazard" to be:

"a product defect which because of the pattern of defect, the number of defective products distributed in commerce, the severity of the risk, or otherwise, creates a substantial risk of injury to the public."

Similarly, EPA has interpreted the word 'substantial' as a quantitative measurement. Thus, a 'substantial risk' is a risk that can be quantified, See, 56 Fed Reg 32292, 32297 (7/15/91). Finally, since information pertinent to the exposure of humans or the environment to chemical substances or mixtures may be obtained by EPA through Sections 8(a) and 8(d) regardless of the degree of potential risk, §8(e) has specialized function. Consequently, information subject to §8(e) reporting should be of a type which would lead a reasonable man to conclude that some type action was required immediately to prevent injury to health or the environment.

Attachment

Comparison:

Reporting triggers found in the 1978 "Statement of Interpretation/ Enforcement Policy", 43 Fed Reg 11110 (3/16/78) and the June 1991 *Section 8(e) Guide*.

<u>TEST TYPE</u>	<u>1978 POLICY CRITERIA EXIST?</u>	<u>New 1991 GUIDE CRITERIA EXIST?</u>
ACUTE LETHALITY		
Oral	N}	Y}
Dermal	N}	Y}
Inhalation (Vapors)	} ⁶	} ⁷
aerosol	N}	Y}
dusts/ particles	N}	Y}
SKIN IRRITATION	N	Y ⁸
SKIN SENSITIZATION (ANIMALS)	N	Y ⁹
EYE IRRITATION	N	Y ¹⁰
SUBCHRONIC (ORAL/DERMAL/INHALATION)	N	Y ¹¹
REPRODUCTION STUDY	N	Y ¹²
DEVELOPMENTAL TOX	Y ¹³	Y ¹⁴

⁶43 Fed Reg at 11114, comment 14:

"This policy statements directs the reporting of specific effects when unknown to the Administrator. Many routine tests are based on a knowledge of toxicity associated with a chemical. unknown effects occurring during such a range test may have to be reported if they are those of concern to the Agency and if the information meets the criteria set forth in Parts V and VII."

⁷Guide at pp.22, 29-31.

⁸Guide at pp-34-36.

⁹Guide at pp-34-36.

¹⁰Guide at pp-34-36.

¹¹Guide at pp-22; 36-37.

¹²Guide at pp-22

¹³43 Fed Reg at 11112

"Birth Defects" listed.

¹⁴Guide at pp-22

NEUROTOXICITY	N	Y ¹⁵
CARCINOGENICITY	Y ¹⁶	Y ¹⁷
MUTAGENICITY		
<i>In Vitro</i>	Y ¹⁸	Y ¹⁹
<i>In Vivo</i>	Y	Y
ENVIRONMENTAL		
Bioaccumulation	Y	N
Bioconcentration	Y ²⁰	N
Oct/water Part. Coeff.	Y	N
Acute Fish	N	N
Acute Daphnia	N	N
Subchronic Fish	N	N
Subchronic Daphnia	N	N
Chronic Fish	N	N
AVIAN		
Acute	N	N
Reproductive	N	N
Reproductive	N	N

¹⁵Guide at pp-23; 33-34.

¹⁶43 Fed Reg at 11112
"Cancer" listed

¹⁷Guide at pp-21.

¹⁸43 Fed Reg at 11112; 11115 at Comment 15

"Mutagenicity" listed/ *in vivo* vs *invitro* discussed; discussion of "Ames test".

¹⁹Guide at pp-23.

²⁰43 Fed Reg at 11112; 11115 at Comment 16.

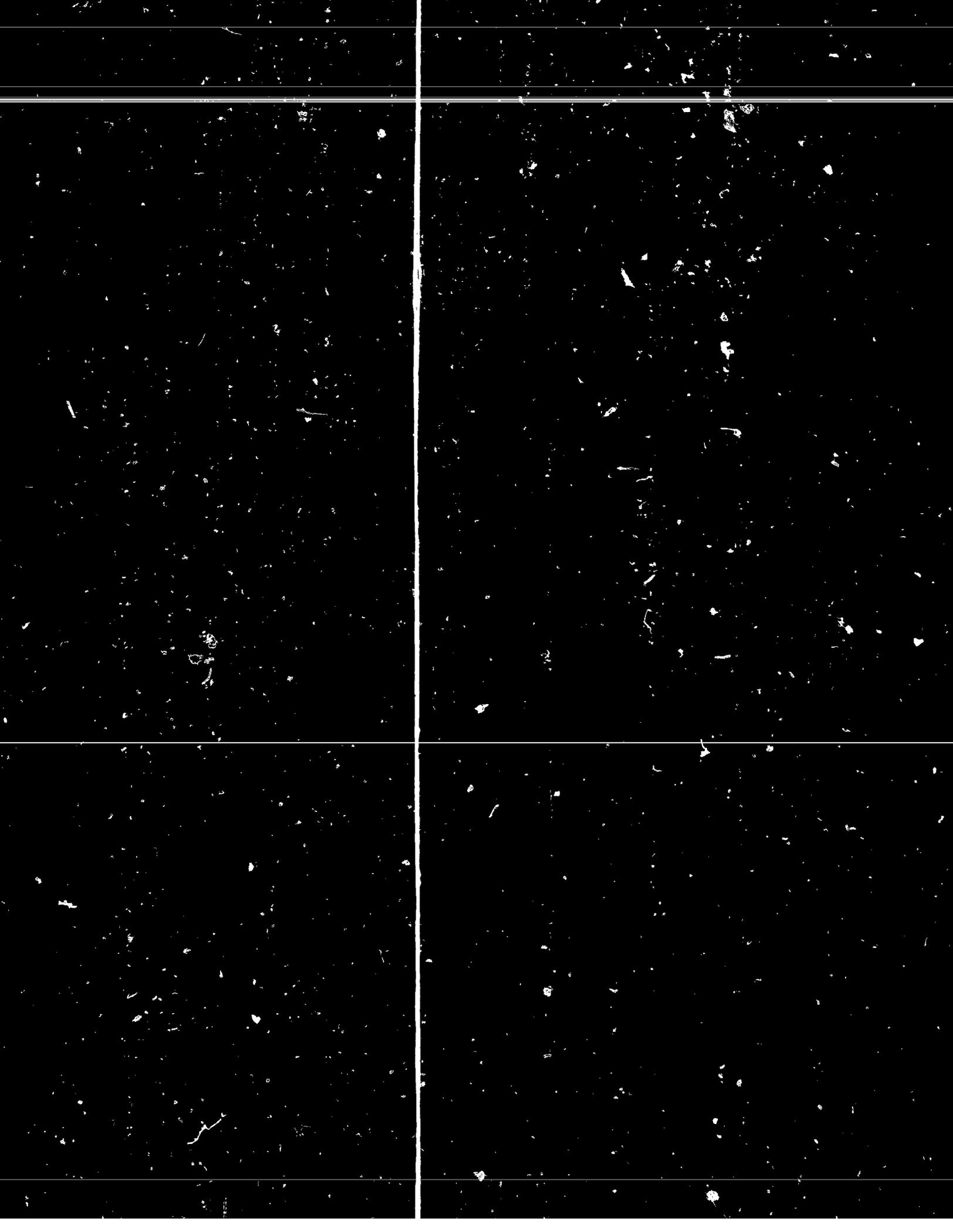
CAS# 110-80-5

Chem: Ethylene Glycol Monoethyl ether

**Title: Ethylene glycol monoethyl ether
Teratogenicity study in rats**

Date: 73/83

Summary of effects: fetotoxicity



37-02

IMPERIAL CHEMICAL INDUSTRIES PLC
CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK, MACCLESFIELD, CHESHIRE, UK

CATEGORY B REPORT (CONFIDENTIAL)
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Sponsor: Chemical Manufacturers *
Association Washington
DC 20037
CTL Ref: Y01733/001
Study No: R0203
Copy No : 14

REPORT NO: CTL/P/761

ETHYLENE GLYCOL MONOETHYL ETHER (EE):
TERATOGENICITY STUDY IN RATS

- D J Tinson
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* This work was carried out for the Glycol Ethers Program Panel of the
CMA under Contract No. GE-3 O-Ter-101.

For Information to CMA
APPROVED FOR PUBLICATION

Approved for Issue: *[Signature]*

Date of Issue

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ETHYLENE GLYCOL MONOETHYL ETHER (EE):
TERATOGENICITY STUDY IN RATS

We, the undersigned, declare that this report constitutes a true record
of the actions taken and the results obtained in the above study.

M. H. Litchfield	Study Director	<i>M. H. Litchfield</i>	31 March '53
D. J. Tinston	Study Investigator	<i>D. J. Tinston</i>	17.3.53
G. A. Wickramaratne	Study Teratologist	<i>G. A. Wickramaratne</i> '53
D. M. Samuels	Study Pathologist	<i>D. M. Samuels</i>	25/3/53
M. J. Gosley	Statistician	<i>M. J. Gosley</i>	25/3/53
L. K. Head	Toxicity Section	<i>L. K. Head</i>	17.3.53
M. T. A. Klimartin	Toxicity Section	<i>M. T. A. Klimartin</i>	17.3.53
M. Killick	Toxicity Section	<i>M. Killick</i>	17.3.53

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CTL/P/751

0013

Central Toxicology Laboratory

Imperial Chemical Industries PLC
Alderley Park
Macclesfield
Cheshire

Study Title: ETHYLENE GLYCOL MONOETHYL ETHER (EE):
TERATOGENICITY STUDY IN RATS

CTL Study Number: RR0203

CTL Report Number: CTL/P/761

The conduct of this study has been inspected/audited by the
Quality Assurance Unit as follows:

Date	Inspection/Audit	Date of QA Report
17 Nov 81	Protocol Audit	17 Nov 81
24 Nov 81	Inspections	24 Nov 81
30 Nov 81	Inspections	30 Nov 81
9 Dec 81	Inspection	9 Dec 81
15 Dec 81	Inspection	15 Dec 81
23 Dec 81	Inspection	23 Dec 81
30 Dec 81	Inspection	4 Jan 82
5 Jan 82	Inspection	5 Jan 82
7 Jan 82	Inspection	11 Jan 82
19 Jan 82	Inspection	19 Jan 82
2 Nov 82	Draft Report Audit	3 Nov 82
6 Apr 83	Final Report Audit	6 Apr 83

Inspection has been carried out and the report has been audited in
accordance with ICI's policies and procedures for Good Laboratory
Practice.

Signature *J.B. Deak*

Date *8th April 1983*

CTL/P/761

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ETHYLENE GLYCOL MONOETHYL ETHER (EE):
TERATOGENICITY STUDY IN RATS

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ETHYLENE GLYCOL MONOETHYL ETHER (EE):
TERATOGENICITY STUDY IN RATS

SUMMARY

Groups of pregnant rats of the Alderley Park (Wistar derived) strain were exposed to 10, 50 and 250ppm of EE for 6 hours per day on days 6-15 (inclusive) of gestation. A concurrent control group was exposed to air only.

There were no effects on maternal body-weight, food consumption or food utilisation at any of the exposure concentrations, and at autopsy on day 21 of gestation there were no maternal macroscopic abnormalities which could be related to treatment. Evidence of maternal toxicity was observed at 250ppm in the form of slight haematological changes.

An increased incidence of intra-uterine deaths, reduced foetal weights and reduced foetal ossification indicative of foetotoxicity were observed in the 250ppm group, but there was no evidence of teratogenicity. There was some evidence for reduced foetal ossification at 50ppm.

It is concluded that EE is not teratogenic to rats at concentrations up to 250ppm, but it is foetotoxic at 250ppm and possibly at 50ppm. There were no toxicologically significant effects at 10ppm.

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1. INTRODUCTION

Ethylene glycol monoethyl ether (EE, 2-ethoxyethanol) is a solvent for celluloses, acrylics, dyes, inks, resins and varnishes. EE has a boiling point of 135°C at 760mm Hg, and a vapour pressure of 4mm Hg at 20°C. Therefore, inhalation could be a major route of exposure for users of this material.

In a previous study reported by Hardin et al, 1981, pregnant Wistar rats were exposed to 767 or 202ppm of EE for 7 hours per day on days 1-19 of gestation. At 767ppm complete embryomortality and maternal toxicity were seen while at 202ppm, foetal growth depression and an increased incidence of terata compared with controls were seen. Thus a no effect level was not established.

The objectives of the study described in this report were to determine a no-effect exposure level of EE for maternal toxicity, embryo/foetotoxicity and teratogenicity for rats exposed during the period of organogenesis i.e days 6-15 of gestation.

The study was sponsored by the Glycol Ethers Program Panel of the Chemical Manufacturers Association, Washington DC.

The study started on the 24 November 1981 and the final post mortem examinations were conducted on the 18 December 1981.

All original data relating to this study are retained in the Archives of Central Toxicology Laboratory and copies of this report are held by the Reports Centre, Central Toxicology Laboratory.

2. EXPERIMENTAL PROCEDURES

2.1 Test Material

EE (>99.9% pure) was assigned a Central Toxicology Laboratory Reference Number Y01733/001 and was supplied by Imperial Chemical Industries PLC.

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Petrochemicals and Plastics Division, Wilton, Middlesbrough, UK, with analytical details. (Appendix 1).

2.2 Animals

Multicarious specific pathogen free, female rats of the Alderley Park strain (Mistar derived) were used since data are available on teratology studies in this strain. The rats had a weight range of 206 to 290g and were approximately 11-13 weeks old on arrival at CTL. Four batches (I-IV) of rats were allocated for mating at the Animal Breeding Unit. The rats in each batch were paired overnight and the following morning the detection of spermatozoa in vaginal smears was used as evidence of mating. Sufficient rats were paired to provide twenty-four impregnated females in each batch. The day on which spermatozoa were detected in the smear was termed Day 0 of pregnancy and on that day the rats were delivered to CTL. The delivery dates were 24 November (Batch I), 25 November (Batch II), 26 November (Batch III) and 27 November 1971 (Batch IV).

2.3 Study Design

The rats were randomly allocated to three exposure groups (250, 50 and 10ppm of EE in air) and one control group (air only) using computer generated random numbers. They were individually identified by a tail tattoo as shown in Table 1.

Table 1

Group No	Exposure Conc'n of EE (ppm)	Total No of rats	Animal Identity Numbers			
			Batch I	Batch II	Batch III	Batch IV
1	0 (Control)	24	1-6	25-30	49-54	73-78
2	10	24	7-12	31-36	55-60	79-84
3	50	24	13-18	37-42	61-66	85-90
4	250	24	19-24	43-48	67-72	91-96

The rats were housed singly in wire mesh cages (dimensions 45 x 21 x 20cm) with removable food hoppers (one per two rats) and water bottles (one per rat). They were offered PC diet and tap water (Appendix 2) ad libitum except during each 6 hour exposure period. The cages were supported in holding chambers, one chamber per group (see 2.4).

Before the first period of exposure, the rats were transferred by cage to exposure chambers of the same design as those used in the pre-exposure period.

On Days 6-15 (inclusive) of gestation the rats were exposed to the appropriate concentration of EE for 6 hours/day.

After the last period of exposure on Day 15 of gestation, the rats were removed by cage from the exposure chambers and placed in holding chambers.

2.4 Exposure and Holding Chambers (Figure 1)

The chambers (Doe and Tinston, 1981) had an internal volume of approximately 3.4m^3 . They were constructed of stainless steel and access was gained to each chamber through a door fitted with a safety glass window.

Air entered at the front of each chamber and was extracted at the back. Within each chamber were six cage levels and excreta collection trays which rotated concurrently (0.5 times per minute) with the direction of flow of the input air. In each chamber, each of the levels supported four cages. The air flow rate through each chamber was set to 600 l/min approximately using a flowmeter (ROTAMETER).

The chamber air supply was conditioned nominally to 22°C and 50% relative humidity, and the temperature and relative humidity in each chamber were recorded daily. (Appendix 3).

The distribution of EE at equilibrium within the chambers was found to be within $\pm 5\%$ of the nominal concentration, as shown in Appendix 4.

2.5 Atmosphere Generation and Analysis

Atmospheres of EE were generated by metering appropriate amounts of EE into a condenser at 35-40°C and passing the vapourised EE into the input air of each chamber (Appendix 5). The concentrations of EE in each chamber were analysed nine to fourteen times per exposure period by means of two infra-red analysers (WILKS-MIRAN) connected to the back wall of the chambers by 4mm id copper tubing. The analysers were set to the following conditions:

10ppm chamber - wavelength 8.9µm, slit width 1mm, path length 10m.

50ppm and 250ppm chambers - wavelength 8.9µm, slit width 1mm, path length 5.5m.

The infra-red analysers were calibrated from chamber concentrations of EE as determined from samples obtained using charcoal tubes (PPA5000). A sample of the chamber atmosphere was drawn through a tube, and the EE was desorbed from the charcoal into chloroform. The amount of EE in the sample was determined by direct injection of the chloroform solution into a gas chromatograph (HEWLETT-PACKARD 5890A) previously calibrated with measured amounts of EE in chloroform. The gas chromatograph oven temperature and flame ionisation detector temperature were maintained at 200 and 350°C, respectively. The detector gases were hydrogen (35ml/min) and air (500ml/min); the carrier gas was nitrogen (30ml/min). A stainless steel column (0.5m long, 4mm id), packed with PORAPAK T (80-100 mesh) was installed.

In addition, daily nominal chamber concentrations were calculated from the weight of EE used to generate each exposure level.

2.6 Maternal Bodyweights, Food Consumption and Clinical Observations

The bodyweight of each rat was recorded on arrival (Day 0), Day 5, Days 6-15 (after exposure), Day 16, Day 18 and Day 21. Food consumption was measured for pairs of females sharing the same hopper on Day 1, daily from Days 6-16 (inclusive), Day 19 and Day 21. Each hopper of food was weighed on the afternoon before these specified times and re-weighed the

following morning. The amount of food left was recorded and the amount of food consumed per rat was calculated. Food wasted was not recorded but was minimal and assumed to be the same for all cages.

Each animal was observed daily for changes in clinical condition and during the exposure period for any abnormalities.

2.7 Terminal Investigations

On day 21 of pregnancy the rats were killed by an overdose of halothane BP (FLUOTHANE, Imperial Chemical Industries PLC) and subjected to a post mortem examination.

Blood samples were taken by cardiac puncture and placed in EDTA pots. The following assays were carried out with a Coulter model S: haemoglobin, haematocrit, total white cell count, red cell count, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration. Blood and bone marrow smears were prepared in the event of any further haematological evaluation but were not subsequently examined. Thymus and spleen were weighed and stored with abnormal tissues in formal saline. These tissues were not subsequently processed further.

The uterus of each rat was dissected out and the gravid uterus was weighed. The number of corpora lutea in each ovary was counted. The uterus was opened by an incision on the abdominal wall and the number of implantations, and the number of early and late intra-uterine deaths were counted. Intra-uterine deaths were identified as being late when foetal tissues were distinguishable. Each live foetus was removed from the uterus by severing the umbilical cord. The foetuses were assigned letters of the alphabet to identify their position in utero starting at the ovarian end of the left horn and ending at the ovarian end of the right horn. Each foetus was then weighed, examined externally for gross abnormalities including cleft palate and identified within the litter by limb tagging.

Approximately half of the foetuses in each litter (randomly selected) were fixed in 70% methanol. These foetuses were subsequently eviscerated

and stained with Alizarin Red S (Staples and Schnell, 1964) for skeletal examination. The remaining fetuses in each litter were fixed in Bouin's fluid to be processed for visceral examination.

2.8 Assessment of Fetuses

2.8.1 Visceral Examinations: During evisceration of the methanol-fixed fetuses the abdominal and thoracic contents were examined and any abnormalities were recorded. At this time the sex of each fetus was determined externally and confirmed by internal examination. Those fetuses fixed in Bouin's fluid were stored until decalcification was complete. They were transferred to 70% methanol. The head and thorax of each fetus was sectioned at approximately 1.5 - 2mm intervals and the sections examined under a stereomicroscope (Wilson, 1965). The abdominal organs were examined in situ and the sex of each fetus was determined. Any abnormalities were recorded.

2.8.2 Skeletal Examinations: The skeletons of all fetuses fixed in methanol and stained with Alizarin Red S were examined under a stereomicroscope to assess morphological development and the degree of ossification. The individual bones of the manus and pes were assessed and the result converted to a four point scale as detailed in Appendix 6.

2.8.3 Classification of Defects: Abnormalities were classified as major (rare or possibly lethal or both) or minor (deviations from normal that are common at external, visceral or skeletal examination) defects. Variations in the degree of ossification of the fetuses were also recorded and classified as minor defects or variants depending on the frequency of occurrence in historical controls. Extra thoracic ribs were classified as variants.

2.9 Statistical Analyses

2.9.1 Maternal Bodyweight, Bodyweight Gain, Food Consumption and Litter Data: Data relating to animals which were not pregnant were not included in the following analyses.

Initial maternal bodyweight, maternal bodyweight gain and food consumption during the pre-exposure, exposure and post-exposure periods

were considered by analysis of variance. In addition, food utilisation during the exposure period was considered by analysis of variance.

Litter data parameters were also considered by analysis of variance. These parameters were: the number of corpora lutea per dam, the number of implantations per foetus, the number of live foetuses, percentage early and late intra-uterine deaths, percentage pre-implantation and percentage post-implantation loss (after double arcsine transformation to stabilise the variance - Freeman and Tukey, 1950), gravid uterus weight, total litter weight (live foetuses) and mean live foetus weight (all calculated on an individual litter basis).

Percentage pre- and post-implantation losses were calculated from the following formulae:

$$\% \text{ pre-implantation loss} = \frac{\text{number of corpora lutea} - \text{number of implantations}}{\text{number of corpora lutea}}$$

$$\% \text{ post-implantation loss} = \frac{(\text{number of implantations} - \text{number of live implantations}) \times 100}{\text{number of implantations}}$$

The analysis of variance allowed for the four different batches of rats supplied for this study. Individual treatment group means were compared with the control group mean using Student's t-test based on the error mean square in the analysis of variance.

Where, for a particular animal, the observed number of corpora lutea was recorded as less than the number of implantations, the number of corpora lutea was assumed to be equal to the number of implantations and the pre-implantation loss assumed to be zero.

In addition to the above analyses, a comparison between each treated group and the control group using Fisher's exact test was also carried out for each of the following parameters - the proportion of animals experiencing any pre-implantation loss, post-implantation loss, early intra-uterine death or late intra-uterine death and the proportion of foetuses which were male.

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All statistical tests of significance used were one-sided except for bodyweight, food consumption, food utilisation, the number of cordons lutei and the proportion of male foetuses, which were two-sided.

2.9.2 Organ Weights and Haematological Parameters: Data relating to animals which were not pregnant were not included in these analyses.

Each haematological parameter, spleen weight and thymus weight were considered by analysis of variance. In addition, the two organ weights were considered by analysis of covariance on final bodyweight.

The analyses allowed for the four different batches of rats supplied for this study. Individual treatment group means were compared with the control group mean using Student's t-test, two-sided, based on the error mean square in the analysis.

2.9.3 Skeletal Data, Visceral and External Foetal Abnormalities: Specific skeletal findings were considered in the following way with the exception of each manus and pes scoring category. The proportion of foetuses in which individual skeletal findings were observed was considered by comparing each treated group with the control group using Fisher's exact test, one-sided. The proportion of foetuses with major or minor skeletal defects and major or minor external and visceral defects were analysed in a similar manner but on a litter basis i.e. the proportion of litters in which, for each of these categories of defect, any foetus was affected.

The mean manus and pes scores and the percentages of foetuses with minor external and visceral defects (after double arcsine transformation Freeman and Tukey, 1950) were considered by analysis of variance. The analysis allowed for the four batches of rats used. Individual treatment group means were compared with the control group mean using Student's t-test, one-sided, based on the error mean square in the analysis.

3. RESULTS

3.1 Atmosphere Analysis (Tables 2 and 3)

The daily mean concentrations of EE in each chamber as determined by

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Infra-red analysis were within 25% of the target concentrations except for the first day of exposure when analyses showed that the 10ppm target was 7.3ppm (-22%) and the 50ppm target was 55.4ppm (-11%) (Table 2).

Nominal concentrations calculated from the rate of use of EE liquid are shown in Table 3. At each exposure level the overall mean nominal concentrations were higher (51% at 10ppm, 9% at 50ppm and 7% at 250ppm) than the overall mean analysed concentrations. The discrepancies probably reflect losses in the generation equipment especially at the 10ppm level, and in addition, there may have been some chamber deposition of EE. The analytical data support the view that the concentrations in the chambers were close to the target levels.

3.2 Maternal Bodyweights, Food Consumption and Clinical Observations (Tables 4 and 5)

There was a statistically significant reduction in the bodyweight gain of the rats exposed to 10ppm of EE during the period of exposure (days 3 to 15) but there were no statistically significant effects in the 50 or 250ppm groups. There were no statistically significant effects on the bodyweight gains of any of the groups exposed to EE during the post-exposure period (days 15 to 21). The overall data show that there was no consistent evidence for an effect due to EE on bodyweight gain.

There was no evidence of any treatment related effects on food consumption or food utilisation in the 50 and 250ppm groups. The higher food utilisation value in the 10ppm group was the result of reduced bodyweight gain noted above and is considered not to be of biological significance.

The incidence of clinical abnormalities was very low. One animal in the 250ppm group had slight hair loss throughout the study and one animal in the 10ppm group had slight piloerection on days 13 to 17 of gestation. There was no evidence that these minor abnormalities had any influence on the pregnancy status of the two dams. There were no changes in clinical condition or behaviour in any of the other rats during the study.

3.3 Terminal Investigations

3.3.1 Maternal Macroscopic Abnormalities (Table 6): The incidence of

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maternal macroscopic abnormalities was higher in the groups exposed to EE than in the controls but there was no evidence that any of these were related to exposure concentration.

3.3.2 Organ Weights (Table 7): There was little evidence of any effect on spleen or thymus weight as a result of exposure to EE.

3.3.3 Haematology (Table 8): There were statistically significant reductions in haemoglobin, haematocrit and mean cell volume in the 250ppm group; other parameters were not affected. There was no evidence of any haematological effects in the 10 and 50ppm groups.

3.3.4 Litter Data (Table 9): There were no inter-group differences in the mean numbers of corpora lutea but mean numbers of implantations were statistically significantly lower in the 10 and 50ppm groups than in the controls. There was, therefore, a higher level of pre-implantation loss in all exposed groups compared with controls although this was only statistically significant in the 10 and 50ppm groups. Post-implantation losses were also slightly higher in the exposed groups compared with controls but these differences were not statistically significant and were within historical control values of 3.9 -16.7% for this strain of rat in this laboratory. The proportion of dams with any late intra-uterine deaths and the mean percentage late intra-uterine deaths were statistically significantly increased in the 250ppm group.

The mean number of live foetuses was reduced in the exposed groups compared with controls. This was statistically significant in the 10 and 50ppm groups but not at 250ppm. The lower litter sizes of the exposed groups were reflected in reduced mean gravid uterus weights and in reduced total litter weights. Gravid uterus weights were statistically significantly reduced in the 10ppm group. Total litter weight was statistically significantly reduced in the 10 and 250ppm groups, the reduction being most marked at 250ppm. Whilst there was no effect on mean foetal weight in the 10 and 50ppm groups, this parameter was statistically significantly reduced in the 250ppm group.

There was no evidence that exposure to EE affected the sex ratios of the foetuses.

3.3.5 Foetal Visceral, External and Skeletal Defects (Tables 10 to 14): The data have been tabulated on a foetal basis and not on a litter basis, but footnotes to the tables indicate any differences between these two approaches to the analysis. The incidence of minor external and visceral defects was slightly elevated in the 10 and 250ppm groups (Tables 10 and 11). The proportion of fetuses affected was statistically significantly higher in the 250ppm group than in the controls, but when considered on a litter basis the increase was not statistically significant. The increases were due to a higher incidence of limb malrotation in the 10ppm group and pelvic dilatation in the 250ppm group. No cardiovascular abnormalities were seen.

There was an increased incidence of minor skeletal defects in the 250ppm group (Table 10), the proportion of fetuses having these defects being statistically significantly increased. Examination of the specific skeletal findings (Table 12) showed this to be the consequence of increased partial/non-ossification of parts of the skull, the thoracic centra, the lumbar centra, the lumbar transverse process and sternbrae, increased sternbral abnormalities and increased incidence of 27 pre-sacral vertebrae. There was little evidence of any increased incidence of these defects in the 10 and 50ppm groups, the only statistically significant increases being the proportion of fetuses with partially ossified lumbar transverse process in the 10ppm group, and partially ossified 2nd sternbrae and unossified cervical centra in the 50ppm group.

The majority of fetuses had one or more skeletal variants and therefore the occurrence of each variant was considered separately. The proportion of fetuses with each specific skeletal variant (except partial ossification of the 5th sternbrae) was statistically significantly higher in the 250ppm group than in the control group. There was also a dose-related increase in the incidence of fetuses with extra ribs and with unossified 1st and 2nd cervical centra.

The development of manus and pes were affected in the 250ppm group, the mean scores per fetus being significantly increased (Table 14). There was no effect on manus and pes in the 10 and 50ppm groups.

4. DISCUSSION

When pregnant rats were exposed to 10, 50 or 250ppm of EE on days 6-15 (inclusive) of gestation there were no treatment-related changes in clinical condition, bodyweight gain, food consumption or food utilisation compared with pregnant rats exposed to air alone. At autopsy on day 21 of gestation the incidence of maternal macroscopic abnormalities in all the groups exposed to EE was higher than controls but there was no evidence that this was related to exposure concentration. Spleen and thymus weights were not affected by exposure to EE. The only evidence of maternal toxicity was in the 250ppm group where statistically significant reductions in haemoglobin, haematocrit and mean cell volume were observed. These changes were slight but are consistent with haematological changes seen in other studies with glycol ethers, including EE (Nagano *et al* 1979, Stenger *et al*, 1971).

Decreased numbers of implantations indicative of a high pre-implantation loss were seen in all exposed groups compared with controls but did not appear to be related to exposure concentration. Control values for percentage pre-implantation loss in several recent studies in this laboratory were 4.3 - 10.8. Thus, the control value of 2.4 in the present study could be considered unusually low and the higher pre-implantation loss in the exposed groups is not toxicologically significant. The slightly increased post-implantation loss observed in the 10 and 50ppm groups is also not toxicologically significant since there were no treatment-related increases in the incidence of intra-uterine deaths in these two groups. However, at 250ppm there was a marked increase in the incidence of late intra-uterine deaths and in the proportion of dams affected thus indicating an increased post-implantation loss. The higher pre-implantation loss and hence the lower number of live fetuses observed in the exposed groups was reflected in reduced gravid uterus weights and total litter weights. There were no differences between the mean live foetal weights in the 10 and 50ppm groups and the controls but the mean foetal weights in the 250ppm group were markedly lower than controls. This would have contributed to the larger reduction in total litter weight observed in the 250ppm group. Thus, 250ppm of EE caused an increased post-implantation loss and retarded foetal growth.

The slight increases in incidences of foetal visceral and external defects in the 10 and 50ppm groups were due to limb malrotation in the 10ppm group and renal pelvis dilatation in the 250ppm group. There were no statistically significant increases in the incidences of limb malrotation in the 50 and 500ppm groups and therefore, this defect has no toxicological significance. The association of renal pelvis dilatation with exposure to 250ppm cannot be precluded, but the defect is a minor one and not indicative of teratogenicity.

No major skeletal defects were identified in the study, but examination of the results for minor defects showed that, overall, there was a foetotoxic effect at 250ppm which was shown by reduced ossification and was probably related to the retarded foetal growth observed at this exposure level. The increased incidence of skeletal variants in the 250ppm group was also consistent with a foetotoxic effect. A small number of the changes occurred at 50ppm viz. unossified cervical centra and extra ribs the incidence of which were just outside the upper end of the historical control ranges (Table 13) and could represent a slight foetotoxic effect. The increased incidence of partially ossified 2nd sternbrae at 50ppm may also be indicative of slight foetotoxicity. Minor changes in ossification of the cervical centra such as those seen at 10ppm are usually reversible once treatment ceases and are therefore of minimal toxicological importance. The incidence of this type of change at 10ppm was in any case within the historical control range for this strain of rat in this laboratory (Table 13) and therefore is not considered to be toxicologically significant. The increased incidence of partially ossified 4th lumbar transverse processes at 10ppm was also seen at 50ppm and is therefore also without toxicological significance.

The results of the study reported here are similar to those reported by Hardin et al (1981) who exposed pregnant female rats to 250ppm of lead for 7 hours a day on days 10 of gestation. Hardin et al found retarded foetal bodyweights accompanied by skeletal defects including retarded ossification. They also found a low incidence of cardio-vascular abnormalities. Six out of 124 foetuses were affected whereas no cardiac abnormalities were seen in the 234 foetuses exposed to 250ppm in this study. The exposure levels used in the two studies were very similar especially as the slightly longer exposure periods (7 hours a day vs

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6 hours per day) in the Hardin et al study are taken into account. It is unlikely that the different length of treatment (days 1-19 of gestation vs days 5-15 of gestation) would have influenced the induction of cardiac abnormalities as the sensitive period of organogenesis is included in both periods. It should be borne in mind that the incidence of cardiac abnormalities in the Hardin et al study (6/324, 1.85%) is low and 200-250ppm may be close to the threshold for this effect in rats.

The data from the present study indicate that EE is not teratogenic to rats exposed during organogenesis at concentrations up to and including 250ppm. It is, however, foetotoxic and shows mild maternal toxicity (haematological changes) at 250ppm and slight foetotoxicity at 50ppm.

5. CONCLUSIONS

When pregnant rats were exposed to 10, 50 and 250ppm of EE for 6 hours per day, on days 5-15 of gestation, foetotoxicity manifested by low foetal weights, reduced ossification and an increased incidence of intra-uterine deaths was observed in the 250ppm group. Evidence for maternal toxicity was also observed only at 250ppm in the form of haematological changes. It was concluded that 250ppm of EE was foetotoxic but not teratogenic and that at 50ppm there was evidence for slight foetotoxicity but there were no toxicologically significant effects at 10ppm.

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