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September 24, 1992

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Washington, DC 20460

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INIT

Attn: Section 8(e) Coordinator (CAP Agreement)

Re: CAP Agreement Identification No. 8ECAP-0110

Dear Sir or Madam:

Union Carbide Corporation ("Union Carbide") herewith submits the following report pursuant to the terms of the TSCA §8(e) Compliance Audit Program and Union Carbide's CAP Agreement dated August 14, 1991 (8ECAP-0110). This report describes a toxicity study with ethylene glycol butyl ether (CASRN 111-76-2) and butoxyacetic acid (CASRN [not available]).

"Ethylene Glycol Butyl Ether and Butoxyacetic Acid: Their Effects on Erythrocyte Fragility in Four Species", ICI (UK), Report No. CTL/T/2256, February 18, 1985. [Also attached to this study are copies of CMA correspondence to the Glycol Ethers Program Panel and TRTG (April 17, 1985, and a CMA letter of April 15, 1985 to EPA with a supplemental filing concerning ethylene glycol monobutyl ether (dated April 11, 1985: "Assessment of Hematologic Toxicity of Ethylene Glycol Monobutyl Ether (EGBE)").

A complete summary of this report is attached.

are: Previous TSCA Section 8(e) or "FYI" Submission(s) related to this substance

ctlt2256

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8/1/95

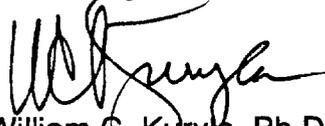
(None)

Previous PMN submissions related to this substance are: (None)

This information is submitted in light of EPA's current guidance. Union Carbide does not necessarily agree that this information reasonably supports the conclusion that the subject chemical presents a substantial risk of injury to health or the environment.

In the attached report the term "CONFIDENTIAL" may appear. This precautionary statement was for internal use at the time of issuance of the report. Confidentiality is hereby waived for purposes of the needs of the Agency in assessing health and safety information. The Agency is advised, however, that the publication rights to the contained information are the property of Union Carbide.

Yours truly,



William C. Kuryla, Ph.D.
Associate Director
Product Safety
(203/794-5230)

WCK/cr

Attachment (3 copies of cover letter, summary, and report)

SUMMARY

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

CATEGORY B REPORT (CONFIDENTIAL)

Division Ref:
CTL Ref: Y00704/003
Y03703/001
CTL Study No: WV 0062
Copy No: 7

REPORT NO: CTL/T/2256

ETHYLENE GLYCOL BUTYL ETHER AND
BUTOXYACETIC ACID : THEIR EFFECTS ON
ERYTHROCYTE FRAGILITY IN FOUR SPECIES

by

P M Hext

This work was supported by Petrochemicals and Plastics Division, Paints Division, ICI Americas and ICI Australia.

THIS DOCUMENT CONTAINS INFORMATION CONFIDENTIAL AND TRADE SECRET TO ICI.

Date of Issue:

18 FEB 1985

SUMMARY

2.

SUMMARY

Erythrocytes prepared from human, rat, dog and rabbit blood samples were incubated with different concentrations of EGBE or butoxyacetic acid.

Rat erythrocytes were unstable in the presence of butoxyacetic acid and lysed at a concentration of 0.05% or greater whereas those of the other species were stable up to 2% concentration. Dog erythrocytes were lysed by EGBE over the entire concentration range employed (0.05 - 0.5%) whereas the stability of the other species was similar in the presence of this compound, all lysing at a concentration of 0.25% or higher.

These results are discussed in relation to in vivo exposure to EGBE and it is concluded that erythrocyte lysis is unlikely to occur in cases of human exposure.

SUMMARY

3.

ASSESSMENT OF HEMATOLOGIC TOXICITY
OF
ETHYLENE GLYCOL MONOBUTYL ETHER (EGBE)

Report prepared for the
Glycol Ethers Panel
of the
Chemical Manufacturers Association
Washington, D. C.

by
Lawrence S. Lessin, M. D.

April 11, 1985

SUMMARY

~~3.~~ 4.

EXECUTIVE SUMMARY

Although the blood and the blood forming organ, the bone marrow, present a multiplicity of potential targets for the toxic effects of ethylene glycol butyl ether (EGBE), the only demonstrated toxicity of this compound in the hematopoietic system of mammalian species is that of accelerated destruction of red blood cells. This effect is most evident in rats and mice in contrast to man whose red cells show resistance to EGBE induced hemolysis. In extensive toxicologic studies carried out in several laboratories from 1943 to 1983, involving mice, rats, rabbits, guinea pigs, dogs, monkeys and human subjects, there has been no evidence for any alteration of the blood cell forming capacity of the bone marrow or the numbers or function of circulating leukocytes or platelets. Alteration of the red blood cell with resultant hemolytic anemia after exposure to EGBE has been the only toxic hematologic effect and this has been confined to a limited number of nonhuman mammalian species. Moreover, a substantial species variation exists in susceptibility to the hemolytic effects of EGBE with the following rank order from most to least susceptible: rat, mouse, rabbit, dog, monkey, guinea pig, man. In rabbits, hemolysis was only seen with subchronic skin applications at high concentrations of EGBE, but not with 13 day subchronic vapor exposures up to 200 ppm. In the guinea pig, no evidence of hemolysis or red cell alteration was found at inhalational exposure levels up to 494 ppm. Human subjects safely tolerated inhalational exposures of EGBE at 195 ppm for up to 8 hours without any hemolytic effect.

SUMMARY

4.

In contrast, the rat and mouse showed unique species specific biochemical susceptibility to the hemolytic effect of EGBE, which is not seen in man or higher mammalian species. The rat showed evidence of red cell alteration following subchronic vapor exposures at levels as low as 54 ppm and frank hemolytic anemia at 200 ppm, whereas mice showed evidence of hemolysis at 100 ppm. The known biochemical and biophysical differences between the red cells of rat and mouse and those of man, render these rodents particularly poor toxicologic models for assessment of potential EGBE hemolytic toxicity in man where a relative resistance to the hemolytic effects of EGBE is evident. Thus, from these toxicologic studies it can be concluded that a hematologically safe subchronic inhalational exposure level for man appears to exceed 200 ppm. Certainly, the current ACGIH level of 25 ppm should provide an ample margin of safety.

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

B-24

CATEGORY B REPORT (CONFIDENTIAL)

Division Ref:
CTL Ref: Y00704/003
Y03703/001
CTL Study No: WV 0062
Copy No: 7

REPORT NO: CTL/T/2256

ETHYLENE GLYCOL BUTYL ETHER AND
BUTOXYACETIC ACID : THEIR EFFECTS ON
ERYTHROCYTE FRAGILITY IN FOUR SPECIES

by

P M Hext

This work was supported by Petrochemicals and Plastics Division, Paints Division, ICI Americas and ICI Australia.

THIS DOCUMENT CONTAINS INFORMATION CONFIDENTIAL AND TRADE SECRET TO ICI.

Approved for Issue:

J. P. Doe
J E Doe

Date of Issue:

18 FEB 1985

ETHYLENE GLYCOL BUTYL ETHER AND BUTOXYACETIC ACID :
THEIR EFFECTS ON ERYTHROCYTE FRAGILITY IN FOUR SPECIES

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

P M Hext
(Study Director)

P M Hext.
.....

11/2/80
.....

ETHYLENE GLYCOL BUTYL ETHER AND BUTOXYACETIC ACID :
THEIR EFFECTS ON ERYTHROCYTE FRAGILITY IN FOUR SPECIES

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INTRODUCTION

One of the major effects seen in toxicity studies carried out with ethylene glycol butyl ether (EGBE) is haematuria and microcytic anaemia caused by the lysis of red blood cells. It is thought that the major metabolite of EGBE, butoxyacetic acid, is responsible for this and a study by Carpenter et al (1956) suggested that the rat may be significantly more sensitive than humans to the effect of these compounds.

The aim of this work was to investigate in greater detail the in vitro results reported by Carpenter et al (1956) which compared the relative haemolytic activities of EGBE and butoxyacetic acid in a number of species.

MATERIALS AND METHODS

Test substances

Ethylene glycol butyl ether (EGBE) was supplied as technical grade material ex BDH Limited and was assigned CTL reference number Y00704/003/002. Butoxyacetic acid was supplied by Biochemical Toxicology Section, Central Toxicology Laboratory and was assigned CTL Reference number Y03703/001/001.

Test solutions

For the initial experiment EGBE and butoxyacetic acid solutions were prepared with veronal buffered saline. For all subsequent experiments solutions were prepared with 0.607% sodium chloride. The pH of all solutions was checked and adjusted to pH7.2 where necessary with sodium hydroxide solution prior to adjustment to the final desired volume with 0.607% sodium chloride.

ETHYLENE GLYCOL BUTYL ETHER AND BUTOXYACETIC ACID :
THEIR EFFECTS ON ERYTHROCYTE FRAGILITY IN FOUR SPECIES

SUMMARY

Erythrocytes prepared from human, rat, dog and rabbit blood samples were incubated with different concentrations of EGBE or butoxyacetic acid.

Rat erythrocytes were unstable in the presence of butoxyacetic acid and lysed at a concentration of 0.05% or greater whereas those of the other species were stable up to 2% concentration. Dog erythrocytes were lysed by EGBE over the entire concentration range employed (0.05 - 0.5%) whereas the stability of the other species was similar in the presence of this compound, all lysing at a concentration of 0.25% or higher.

These results are discussed in relation to in vivo exposure to EGBE and it is concluded that erythrocyte lysis is unlikely to occur in cases of human exposure.

RESULTS

The initial experiment was designed to measure the haemolytic activity of EGBE at concentrations of 2.5% and 1%, and butoxyacetic acid at concentrations of 0.2% and 0.1%, towards rat and human erythrocytes. The conditions employed were based upon those used to assess the haemolytic activity of particulates (Desai, Hext and Richards; 1975). Results from this experiment using butoxyacetic acid (Table 1) showed no haemolysis with human erythrocytes, this being less than that observed with the fragility control. In those samples incubated with rat erythrocytes there was a marked haemolysis (38.4% compared with totally lysed sample) in the fragility controls with less haemolysis in the test samples.

All samples containing EGBE turned brown upon addition of erythrocytes. This problem was found to be caused by incomplete solubilisation of EGBE at these concentrations which resulted in a slow but measureable decrease in pH in these solutions after the initial adjustment to pH7.2. This problem was only overcome by employing 0.5% EGBE as the maximum permissible concentration for incubations.

Having demonstrated that the conditions used for the testing of the haemolytic potential of particulates appeared unsuitable for this study, the conditions employed by Carpenter et al (1956), namely 0.68% sodium chloride and 37°C incubation, were adopted. Table 2 shows the haemolytic activity of a wide range of butoxyacetic acid concentrations incubated with erythrocytes from all four species. It is apparent that only rat erythrocytes readily lysed and that there was a very marked increase in haemolysis with small increase in concentration around 0.05%. This can be seen more clearly in Figure 1 where the haemolysis of rat and human erythrocytes are compared.

The lower haemolysis figure with rat erythrocytes at 2% butoxyacetic acid may represent osmotic protection afforded by using such a high concentration of this compound.

Blood samples

Human, dog and rabbit blood samples were taken by venupuncture. Rat blood samples were taken by cardiac puncture following deep anaesthesia with halothane BP (FLUOTHANE, Imperial Chemical Industries PLC, Pharmaceuticals Division).

All blood samples were of approximately 10ml volume and were transferred to lithium heparin coated tubes to prevent coagulation. After gentle mixing each sample was processed to produce a washed and standardised erythrocyte suspension in veronal buffered isotonic saline.

Haemolysis testing

In all experiments 1ml of erythrocyte suspension was added to 3ml of the desired test or standard solution. The suspension was then gently mixed for 1h followed by centrifugation at 2000rpm at room temperature in a bench centrifuge for 10min. The extinction of the supernatant was measured at 541nm in a Pye Unicam SP6-500 spectrophotometer and the extinction compared with that of a totally lysed sample (water replacing test solution) and a fragility control (no test substance in solution). The haemolytic activity was calculated using the following formula:

$$\% \text{ Haemolysis} = 100 \times \frac{\text{Extinction of test} - \text{Extinction of fragility control}}{\text{Extinction of totally lysed erythrocytes}}$$

The initial experiment used veronal buffered solutions under isotonic saline conditions with mixing in stoppered plastic tubes at room temperature.

All remaining experiments reported here employed overall sodium chloride concentrations of 0.68% (1ml erythrocytes in isotonic saline added to 3ml of test solution prepared with 0.607% sodium chloride gives an overall concentration of 0.68%) with shaking at 37°C in stoppered quickfit 25ml conical flasks. After shaking, the flask contents were transferred to 10ml plastic tubes for the centrifuge stage.

(Table 3)
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resistant to such an effect over a wide range of concentrations, dog erythrocytes showing a slight haemolysis at one of the lower concentrations. When erythrocytes were exposed to EGBE a different pattern of effects was evident. Rat, human and rabbit erythrocytes were lysed at similar concentrations (0.25% - 0.5%) whereas at all concentrations (0.05% - 0.5%) a high percentage of those from the dog lysed.

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If results from this study are comparable in any way with the in vivo effects of exposure to EGBE, the absence of haemolytic activity in the dog suggests that little, if any, of the parent compound reaches the erythrocyte. Similarly, on the basis of EGBE alone, the rat should be no more sensitive than the human or rabbit. However, since it is the results with butoxyacetic acid that correlate very strongly with the haemolytic effect seen in vivo in different species upon exposure to EGBE, the very marked sensitivity of the rat in vivo to EGBE and rat erythrocytes in vitro to butoxyacetic acid suggest that it is the latter compound, a metabolite of EGBE, that is responsible for these effects. The marked stability of human erythrocytes when incubated with this compound over a wide range of concentrations, together with the limited data on human exposure to EGBE, support the theory that the rat is significantly more sensitive than humans to EGBE exposure, and that erythrocyte lysis is unlikely to occur in cases of human exposure.

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Incubation of erythrocytes with increasing concentrations of EGBE (Table 3) demonstrated that there was little difference between the rat, human and rabbit with respect to the concentration required to produce marked haemolysis, whereas dog erythrocytes appeared to be very unstable in the presence of EGBE at all concentrations employed.

DISCUSSION

The work reported here was designed to further substantiate the conclusions of Carpenter et al (1956) which were derived from results using a very arbitrary end point for comparing the haemolysis of erythrocytes from a number of species in the presence of EGBE or butoxyacetic acid. The different methodologies employed in the two studies make it difficult to readily compare the two sets of results, nonetheless, there is very good correlation between the main conclusions drawn from both studies.

It is apparent from the conditions employed by Carpenter et al (1956) and later adopted in this study, that any haemolytic activity of the compounds tested depends upon the erythrocytes being subjected to slight stress. In these studies this was applied by using hypotonic conditions whereas in vivo there might be mechanical stress during passage of the cells through the fine blood capillaries. Both types of stress would make erythrocytes more susceptible to membrane damage. If this subsequently occurs as a result of exposure to the test compound, it would be apparent in vitro as release of haemoglobin into the medium and in vivo as haematuria.

The major aim of this study was to compare the in vitro haemolysis of human and rat erythrocytes in the presence of butoxyacetic acid and EGBE. This was extended to include two other species, the rabbit and the dog, which in vivo do not appear to develop haematuria following exposure to EGBE. It can be seen from the results that of the four species tested here, rat erythrocytes appear to be very susceptible to damage (resulting in lysis by butoxyacetic acid. Human, dog and rabbit erythrocytes were

ETHYLENE GLYCOL BUTYL ETHER AND BUTOXYACETIC ACID :
THEIR EFFECTS ON ERYTHROCYTE FRAGILITY IN FOUR SPECIES

TABLE 1

EXTINCTION AT 541nm OF SUPERNATANTS FROM HUMAN AND
RAT ERYTHROCYTES INCUBATED AT ROOM TEMPERATURE IN
VERONAL BUFFERED ISOTONIC SALINE ALONE OR CONTAINING
0.1% AND 0.2% BUTOXYACETIC ACID

Species	Extinction at 541nm		
	0.1% Butoxyacetic acid	0.2% Butoxyacetic acid	Fragility Control
Human	0.012	0.011	0.025
Rat	0.170	0.139	0.217

REFERENCE

Carpenter C P, Pozzani U C, Weil C S, Nair J H, Keck G A and Smyth H F
Jnr (1956). Arch Ind Hlth 14, 114-131.

Desai R, Hext P M and Richards R J (1975). Life Sci 16, 1931-1938.

ETHYLENE GLYCOL BUTYL ETHER AND BUTOXYACETIC ACID :
THEIR EFFECTS ON ERYTHROCYTE FRAGILITY IN FOUR SPECIES

TABLE 3

HAEMOLYTIC ACTIVITY OF VARIOUS CONCENTRATIONS OF EGBE
INCUBATED WITH ERYTHROCYTES FROM RAT, HUMAN, DOG AND RABBIT

Species	Haemolysis (%)				
	Overall Concentration (%)				
	0.05	0.1	0.25	0.4	0.5
Rat	-	0.0	2.5	51.5	62.0
Human	-	0.0	1.5	20.5	70.9
Dog	46.8	36.2	-	41.2	62.3
Rabbit	-	0.0	2.8	83.7	72.0

Each figure is the mean of at least duplicate determinations unless one of the duplicates gave 0.0% haemolysis.

ETHYLENE GLYCOL ETHER AND BUTOXYACETIC ACID :
THEIR EFFECTS ON ERYTHROCYTE FRAGILITY IN FOUR SPECIES

TABLE 2

HAEMOLYTIC ACTIVITY OF VARIOUS CONCENTRATIONS OF
BUTOXYACETIC ACID INCUBATED WITH ERYTHROCYTES FROM
RAT, HUMAN, DOG AND RABBIT

Species	Haemolysis (%)								
	Overall Concentration (%)								
	0.01	0.02	0.03	0.04	0.05	0.1	0.2	0.5	2.0
Rat	0.8	4.4	8.4	11.1	53.0	91.9	97.9	98.8	24.9
Human	0.8	1.4	-	-	0.2	0.0	1.2	0.8	0.0+
Dog	0.0	13.6	-	-	0.0	0.0	0.0	0.0	2.6+
Rabbit	4.9	0.0	-	-	3.2	1.2	0.0	1.1	1.7

Each figure is the mean of at least duplicate determinations unless one of the duplicates gave 0.0% haemolysis or those marked + where one of the duplicates was lost.

ETHYLENE GLYCOL BUTYL ETHER AND BUTOXYACETIC ACID :
THEIR EFFECTS ON ERYTHROCYTE FRAGILITY IN FOUR SPECIES

CIRCULATION

Internal

- 1 Report Centre Reference Copy
- 2 Report Centre - spare
- 3 Dr I F H Purchase)
Dr S E Jagers .)
Dr R S Morrod)
- 4 Dr J E Doe
- 5 Dr P M Hext

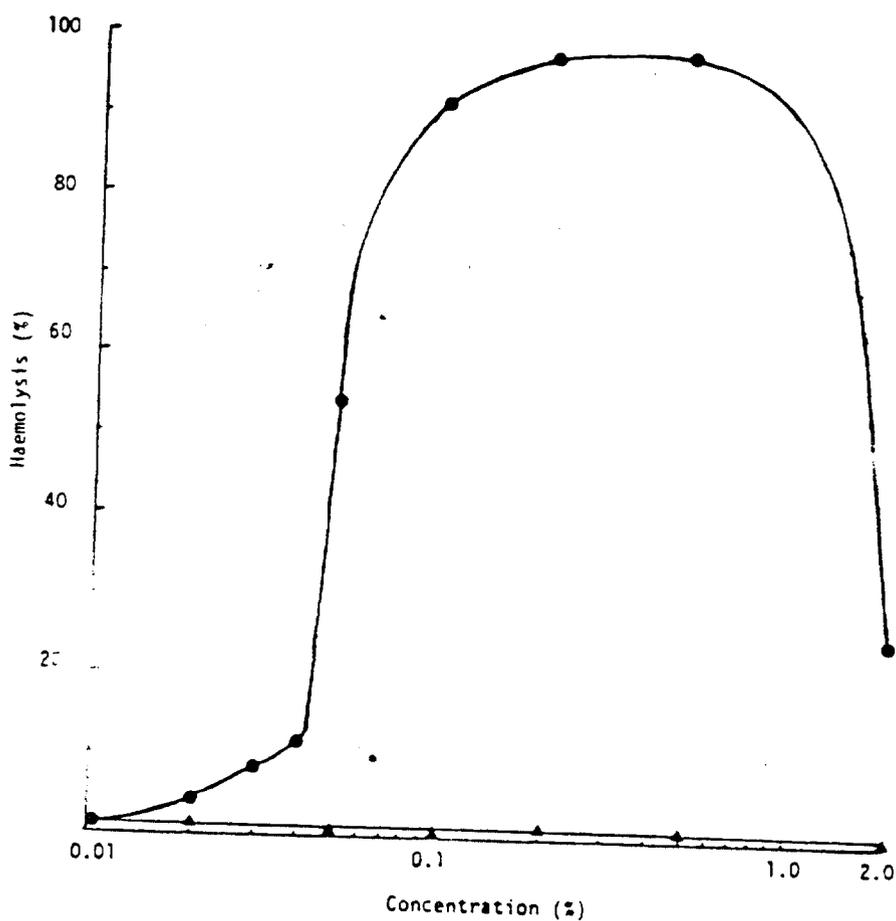
External

- 6 Mr P Anderson, PC and P Division
- 7 Dr J P Lyon, ICI, US
- 8 Dr T D Brown, Paints Division
- 9 Dr A Parsons, ICI Australia

ETHYLENE GLYCOL BUTYL ETHER AND BUTOXYACETIC ACID :
THEIR EFFECTS ON ERYTHROCYTE FRAGILITY IN FOUR SPECIES

FIGURE 1

HAEMOLYTIC ACTIVITY OF VARIOUS CONCENTRATIONS OF BUTOXYACETIC ACID
INCUBATED WITH ERYTHROCYTES FROM RAT (●-●) AND HUMAN (▲-▲)





CHEMICAL MANUFACTURERS ASSOCIATION

April 17, 1985

To: Glycol Ethers Program Panel and TRTG

From: C. Stack *C. Stack*

Re: Lessin Report

The final report from Dr. Lessin on his review of EGBE hematotoxicity is enclosed along with the transmittal letters to EPA. The Lessin review, and the ICI study report referred to in the letter addressed to Harry Teitelbaum, were sent to EPA on April 15.

ASSESSMENT OF HEMATOTOXICITY OF
ETHYLENE GLYCOL MONOBUTYL ETHER (EGBE)

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 - 1.2.1 Effects on the RBC membrane
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- IV. Hemolytic susceptibility of the rat red blood cell
- V. Laboratory assessment of hemolysis



CHEMICAL MANUFACTURERS ASSOCIATION

April 15, 1985

Ms. Joni T. Repasch
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Room 106, East Tower
Washington, D. C. 20460

Re: 2-2-(1-methoxyethoxy) Ethyl Acetate ANPR,
OPTS 2062; Supplemental Filing

Dear Ms. Repasch:

When the Chemical Manufacturers Association Glycol Ethers Panel filed on February 19, 1985 its ANPR comments on the Agency's consideration of a TSCA Section 4 test rule for diethylene glycol butyl ether (DGBE) and its acetate (DGBA), we discussed the preliminary conclusions of Dr. Lawrence Lessin on studies of hematologic effects of EGBE (at pp. 24-25). Dr. Lessin has now completed his assessment, and we enclose a copy of his report for the record, as well as a letter to Dr. Harry Teitelbaum summarizing the report.

Sincerely,

Carol Stack, Ph.D.
Manager, Glycol Ethers
Program Panel

Enclosures

cc: Mr. Frank Benvenuti



CHEMICAL MANUFACTURERS ASSOCIATION

April 15, 1985

Dr. Harry Teitelbaum
Existing Chemicals Control Branch
Chemical Control Division
Office of Toxic Substances
Environmental Protection Agency
401 M Street, S.W.
Room 529-A, East Tower
Washington, D.C. 20460

Dear Dr. Teitelbaum:

On April 26, 1984, the CMA Glycol Ethers Panel sent you a preliminary review of the many studies of red blood cell effects of ethylene glycol monobutyl ether (EGBE). Since that time, the Panel has continued to review these data and has consulted with Dr. Lawrence S. Lessin, Chairman of the Department of Hematology at the George Washington University Medical School, concerning their interpretation. Dr. Lessin has just completed his assessment of the hematologic toxicity of EGBE. I am enclosing a copy of his report to assist EPA in its continuing review.

Based on assessment of more than 24 hematologic studies of EGBE, Dr. Lessin finds (at p. 2) that "a hematologically safe subchronic inhalational exposure level for man appears to exceed 200 ppm." Thus, he concludes: "Certainly, the current ACGIH level of 25 ppm should provide an ample margin of safety." Id.

Dr. Lessin's assessment provides both a background discussion of hematologic effects that can be caused by chemical exposure and a detailed discussion of the EGBE data. He notes (at p. 1) that the "only toxic hematologic effect" of EGBE that has been found in any species is "alteration of the red blood cell with resultant hemolytic anemia." This effect, he notes further, "has been confined to a limited number of nonhuman mammalian species" (id.) and has been found at exposures less than 200 ppm only in the rat and mouse (see

Dr. Harry Teitelbaum

Page 2

April 15, 1965

Dr. Lessin's appended graphs). Dr. Lessin notes, however, (at p. 2) that "known biochemical and biophysical differences between the red cells of rat and mouse and those of man" cause these rodents to be "particularly poor toxicologic models for assessment of potential EGBE hemolytic toxicity in man."

Dr. Lessin further notes that human data on EGBE hematologic effects confirm the unsuitability of rat and mouse results for determining safe exposure levels for man. Calling the relative resistance to hemolytic effects of man "evident" (at p.2) and noting that human studies found no effects with exposures as high as 195 ppm, Dr. Lessin concludes human hemolysis susceptibility would be better predicted by rabbit or guinea pig results that are much more likely to be "similar to that of man" (at p.19). Inasmuch as both of these species have evidenced no hemolytic effects at exposures up to 494 ppm, Dr. Lessin concludes (at p.2) that the experimentally derived human no-effect level of 200 ppm can confidently be found to "exceed 200 ppm."

The Panel is also enclosing for your information the report of a study of in vitro blood effects, "Ethylene Glycol Butyl Ether and Butoxyacetic Acid: Their Effects on Erythrocyte Fragility in Four Species," that was recently conducted by Imperial Chemical Industries in the United Kingdom. This report provides additional data supplementing the conclusions reached by Dr. Lessin. Although the cover page characterizes the ICI study as confidential, we have been assured by ICI that it need not be handled as such.

The Panel hopes Dr. Lessin's expert review of the hematologic data on EGBE will be useful to the Agency. If you have any questions, please feel free to call me.

Sincerely,

Carol Stack

Carol Stack, Ph.D.
Manager, Glycol Ethers
Program Panel

Enclosure

ASSESSMENT OF HEMATOLOGIC TOXICITY
OF
ETHYLENE GLYCOL MONOBUTYL ETHER (EGBE)

Report prepared for the
Glycol Ethers Panel
of the
Chemical Manufacturers Association
Washington, D. C.

by
Lawrence S. Lessin, M. D.

April 11, 1985

EXECUTIVE SUMMARY

Although the blood and the blood forming organ, the bone marrow, present a multiplicity of potential targets for the toxic effects of ethylene glycol butyl ether (EGBE), the only demonstrated toxicity of this compound in the hematopoietic system of mammalian species is that of accelerated destruction of red blood cells. This effect is most evident in rats and mice in contrast to man whose red cells show resistance to EGBE induced hemolysis. In extensive toxicologic studies carried out in several laboratories from 1943 to 1983, involving mice, rats, rabbits, guinea pigs, dogs, monkeys and human subjects, there has been no evidence for any alteration of the blood cell forming capacity of the bone marrow or the numbers or function of circulating leukocytes or platelets. Alteration of the red blood cell with resultant hemolytic anemia after exposure to EGBE has been the only toxic hematologic effect and this has been confined to a limited number of nonhuman mammalian species. Moreover, a substantial species variation exists in susceptibility to the hemolytic effects of EGBE with the following rank order from most to least susceptible: rat, mouse, rabbit, dog, monkey, guinea pig, man. In rabbits, hemolysis was only seen with subchronic skin applications at high concentrations of EGBE, but not with 13 day subchronic vapor exposures up to 200 ppm. In the guinea pig, no evidence of hemolysis or red cell alteration was found at inhalational exposure levels up to 494 ppm. Human subjects safely tolerated inhalational exposures of EGBE at 195 ppm for up to 8 hours without any hemolytic effect.

In contrast, the rat and mouse showed unique species specific biochemical susceptibility to the hemolytic effect of EGBE, which is not seen in man or higher mammalian species. The rat showed evidence of red cell alteration following subchronic vapor exposures at levels as low as 54 ppm and frank hemolytic anemia at 200 ppm, whereas mice showed evidence of hemolysis at 100 ppm. The known biochemical and biophysical differences between the red cells of rat and mouse and those of man, render these rodents particularly poor toxicologic models for assessment of potential EGBE hemolytic toxicity in man where a relative resistance to the hemolytic effects of EGBE is evident. Thus, from these toxicologic studies it can be concluded that a hematologically safe subchronic inhalational exposure level for man appears to exceed 200 ppm. Certainly, the current ACGIH level of 25 ppm should provide an ample margin of safety.

1.0 Blood and bone marrow as target organs for chemotoxicity.

The cellular elements of the circulating blood and their precursor cells in the bone marrow may be considered as a single hematopoietic organ. Because of the highly proliferative state of blood forming cells in the marrow and the relatively short survival and metabolic dependence of peripheral blood elements, the hematopoietic organ is a susceptible target for the toxic effects of chemical agents. Ease of access to blood and relative simplicity of detection of hematotoxic effects in test animals and man makes the hematopoietic system a convenient marker target organ for assessment of toxicity of a broad spectrum of compounds.

1.1 Hematopoietic targets for chemotoxicity. Normal function of hematopoiesis is critically dependent upon the integrity of the structure and chemical composition of its ground substance or "soil" and cellular element or "seed." Furthermore, the environmental and chemical requirements for the constant proliferation and renewal of erythroid, myeloid and thrombopoietic lines and their responsiveness to peripheral demands and stimuli, place hematopoiesis in a position particularly vulnerable to chemical insult. Thus, direct chemical damage to the "soil" by oxidation, liganding, intercalation, crosslinking or induced release of hydrolytic enzymes will impair its support of an inductive microenvironment. Similarly, direct chemical insult to DNA synthesis or repair, protein synthesis, mitochondrial energy production or lipid membrane biosynthesis risks retardation or inhibition of the

necessary rapidly responsive proliferation of any or all of the three cell lines. Chemical injury to hematopoiesis may also take the form of damage to or inhibition of a necessary metabolite. Such methods are exploited in therapeutic attempts to control or abolish growth of cancer cells and almost always result in marrow injury, in a predictable dose-response fashion. If the inhibitory chemical agent has not been given in excessive dose or time of exposure, hematopoietic recovery usually ensues within 10-21 days. Repeated insults of this type, particularly when coupled with the additive effects of radiation, may deplete the hematopoietic stem cell pool and impair subsequent marrow recovery. In most cases however, oncologists have learned how to use chemotherapeutic compounds such that cancer cells are selectively killed and marrow recovery ensues.

Chemical marrow injury may also occur by direct toxicity to mitochondrial energy production or energy-dependent pathways. For example, lead deactivates several enzymes in the heme-synthetic pathway, blocking erythropoiesis. Isoniazide antagonizes the action of vitamin B6, essential to heme synthesis. Ethanol, in concentrations over 100mg/dL also inhibits this pathway by preventing high-energy phosphorylation of vitamin B6.

Cellular protein synthesis is inhibited by agents such as chloramphenicol and asparaginase, leading to cell death due to

growth failure. Such agents deprive marrow cells of structural and enzymatic proteins critical to continued maturation, amplification and production of their cellular end products. The resultant pathologic process is termed ineffective hematopoiesis, usually accompanied by production failure and premature death of marrow precursor cells.

Hematopoiesis. In mammalian species hematopoiesis occurs largely within the marrow spaces of porous bones of the axial skeleton, although in some rodents, the spleen and liver are sites of hematopoiesis. The formation of all blood cells derives from a single multipotential progenitor cell, the "stem" cell. Under appropriate stimuli and control, the stem cell gives rise to red cells, leukocytes and platelets through a series of cell divisions which permit both maturation and amplification. Thus a single stem cell can potentially give rise to numerous mature red blood cells, leukocytes or platelets.

1.1.1 Erythropoiesis. Under the stimulus of the hormone, erythropoietin, the stem cell commits to erythropoiesis, which is the formation of red cells. This hormone is produced by the kidney when a demand for increased red cell production is created by blood loss or hemolysis, the premature destruction of red cells. Over an average period of 72 hours the human stem cell matures through the developing erythroblasts to the reticulocyte which enters the circulation as a "newborn" red cell. The red cell then circulates for approximately 120 days under normal conditions in man and somewhat shorter periods in lower mammalian species.

Any alteration in the red cell or its circulatory environment, results in a hemolytic state, which is usually partially

compensated by acceleration of erythropoiesis. (The principal and only toxic effect of EGBE on the blood appears to be such an alteration of the circulating RBC and only in selected non-human mammalian species. See section 2.0, below.) Under normal conditions about 1% of our red cells are destroyed and replaced each day. In a clinical hemolytic anemia 20% or more of the red cell mass may be destroyed in a day, exceeding the marrow capacity for replacement.

1.1.2 Granulopoiesis and immunoproliferation. White blood cell (WBC) formation in the bone marrow may be simplified into two principal compartments, granulopoiesis--the production of the granulated leukocytes--and immunoproliferation--the formation of lymphocytes and plasma cells. Granulocytes recognize, engulf and kill bacteria and other "foreign" invaders and the immunocytes, lymphocytes and plasma cell are responsible for cellular immune response and antibody production. Granulopoiesis proceeds from the multipotential stem cell to a committed precursor cell capable of producing only granulocytes and monocytes.

Initiation and acceleration of this type of white blood cell formation occurs in response to peripheral signals which indicate bacterial infection or tissue injury. When this occurs, a stepwise sequence of maturation and amplification proceeds from stem cell to myeloblast to each of the granulocytic precursor stages, resulting in numerous mature granulocytes formed from a single stem cell in a period of 3-5 days. Usually when this occurs, as with bacterial infection, a rise in the peripheral white blood cell count is seen along with the presence of less mature granulocytes called juvenile or band forms. Such a change in the white blood cell count is often

used diagnostically by physicians. In some of the EGBE toxicity studies discussed below, inconsistent elevation of WBC counts in test animals (but not in human subjects) appeared to be due to experimental stress, tissue injury or concurrent bacterial infection.

The production and control of the immunocytes--lymphocytes and plasma cells is not confined to the marrow but exists in lymph nodes, spleen, and in multiple organ sites, often close to where the cellular immune response or antibody effect is required. The marrow however, is a major site of lymphocyte and plasma cell formation. There, these cells respond to a variety of antigenic stimuli, by proliferation, antibody production and the release of signals which influence the function of immunocytes both locally and elsewhere in the body. These cells also derive from the basic stem cell and differentiate into a series of specific forms with specialized functions such as "helper, suppressor and killer-" T-cells and antibody producing B-lymphocytes and plasma cells. Immunocytes and their production pathway are quite susceptible to toxic "immunosuppression" by a variety of chemical and physical agents, among which the agents used to treat cancer such as drugs which inhibit DNA synthesis or ionizing radiation. No evidence exists for any such effect of EGBE on the immune system.

1.1.3 Thrombopoiesis. Platelet production is carried out by the megakaryocytes of the bone marrow. As with the other marrow elements, these cells derive from the stem cell and undergo nuclear division without cell division, resulting in very large cells with multiple nuclei and vast cytoplasm. These cells are virtual platelet factories which form platelet elements in

their interiors and bud formed platelets from their surfaces often releasing the platelets directly into the circulating blood. No qualitative or quantitative alterations of platelets or their production was observed in any EGBE toxicity studies reported.

1.2 Peripheral blood cells as targets for chemotoxicity.

Because of their specific structural constituents and metabolic requirements the RBCs, granulocytes (WBCs) and platelets (thrombocytes or TBCs) present unique targets for the chemotoxic effects of offending agents. As suggested earlier, the ease of access to blood as a biologic specimen and the relative simplicity of measuring hematotoxic effects, tends to make blood a focus for toxicologic monitoring. At the same time, alterations in numbers and functional state of peripheral blood elements, can be a toxicologic "tip of the iceberg" and herald damage to other organs or structures more difficult to assess. On the contrary, the unique characteristics of e.g., the RBC or even the RBC of a single species, may render such cells selectively susceptible to the chemo-toxicity of a given compound. RBCs, the only peripheral blood element shown to be consistently affected in any species by EGBE will be discussed in detail below. Toxic alterations of leukocytes and platelets (not found with EGBE) are described in Appendix III.

1.2.1 Mechanisms of red blood cell chemotoxicity.

The reticulocyte emerges from the marrow within 48 hours of its production and spends the first 24 hours of its circulatory lifespan undergoing remodelling during repetitive passage through the spleen and microcirculation. In this process this new RBC loses excess

membrane and unneeded organelles and assumes the biconcave disc shape of the mature erythrocyte, approximately 8 by 2.5 cubic microns in size. As such, the mature RBC survives for 100-120 days in the circulation during which it travels an average of 175 miles. To accomplish this it must maintain its deformability which is linked to the biochemical integrity of the cell membrane, its cation and water content, metabolic pathways and hemoglobin.

Because of the 100 day lifespan of the RBC, about 1% of the RBC mass is normally lost and replaced each day. Thus, about 1% of RBCs can be identified as reticulocytes; when the rate of RBC destruction or loss is increased, the erythropoietic rate accelerates and the reticulocyte percentage increases in proportion to the degree of shortening of RBC survival. The peripheral blood reticulocyte count, therefore, is a direct reflection of the erythropoietic response. Any chemical agent which affects the structural or biochemical integrity of the RBC will decrease its deformability and thus shorten its survival, provoking a compensatory erythropoietic response.

1.2.1.1 The RBC membrane. The RBC is enveloped by a membrane consisting of a protein matrix and cytoskeleton interwoven with a lipid bilayer. The latter is composed of phospholipids oriented with hydrophilic portions toward the cell exterior and cytoplasm and hydrophobic ends within the membrane interior; cholesterol saturates 40% of its binding sites among the phospholipid moieties. Proteins are located on both the exterior and interior membrane surfaces; some traverse the entire thickness of the bilayer. Exterior protein projections bear negative charges and the oligosaccharide antigens of the ABO and related blood groups. Proteins on the membrane interior are organized into a cytoskeleton network comprised primarily of alpha and beta spectrin,

f-actin, ankyrin, protein 4.1 attached to the interior projection of Band-3, a major transmembrane protein which also serves as the sodium-potassium transport pore. The specific interprotein linkages within the cytoskeleton are chemically vulnerable and essential to normal RBC shape and deformability. Lipophilic chemical agents may enter the lipid bilayer and alter its balance of forces and fluidity. Detergents attack the bilayer, causing outward bending, and echinocytic (spiculated) shape change. Amphophilic compounds such as phenothiazines enter the inner membrane leaflet, causing inward membrane folding or stomatocytic (bowl) shape alteration. Lipases directly cleave phospholipid moieties and produce rapid RBC lysis.

Chemical damage to the cytoskeleton may be induced by oxidant or crosslinking agents. Such agents tend to "freeze" the membrane and render it undeformable. Oxidant agents are those which generate hydrogen peroxide, hydroxyl radical and superoxide. Protein crosslinkage is induced by sulfhydryl agents, and compounds initiating abnormal hydrogen and covalent bonds. RBC energy depletion resulting in lack of high energy ATP permits rigidification of the cytoskeleton due to excess binding of calcium.

1.2.1.2 RBC metabolic pathways. The metabolism of the RBC may be simplified by focusing upon its products: ATP, necessary to fuel the cation transport mechanisms of the cell and to phosphorylate essential proteins; reduced glutathione (GSH) the major intracellular reducing agent protective against damage to hemoglobin and other proteins by oxidant radicals; 2,3-diphosphoglycerate (2,3-DPG) which enhances oxygen release from

hemoglobin to the tissues. ATP is the product of the glycolytic pathway which metabolizes glucose into 3-carbon fragments and eventually lactate, with a net yield of 4 ATP per glucose. GSH along with NADPH are the principal products of the hexose-monophosphate shunt, catalyzed by G6PD (glucose-6-phosphate dehydrogenase) and linked to the glutathione reductase and peroxidase reactions. 2,3-DPG is produced in the Leubering-Rappaport shunt from the glycolytic pathway, catalyzed by DPG mutase.

Each of these three major RBC metabolic pathways can be chemically antagonized. The best example and most frequently encountered situation in man is that of oxidant-induced hemolysis due to a mutant G6PD enzyme resulting in lack of adequate GSH to prevent hemoglobin oxidation. This example is of particular interest since the pattern of hemolytic anemia is similar to that induced by EGBE in some rodent species (but not in humans). G6PD is a polymorphic enzyme with over 100 variants described. The normal caucasian variant is GdB+ and black variant is GdA+. Ten percent of black males have the deficient GdA- variant; 0.5 % or less of caucasians have the GdB- variant. Usually, only males are affected because the trait is of sex-linked inheritance. When individuals with GdA- or GdB- are exposed to oxidant generating drugs (e.g., primaquine, sulfonamides) or chemicals (e.g., naphthylalene, fava beans) brisk hemolysis marked by hemoglobinuria, fall in hematocrit and rise in reticulocyte count occurs. In GdA- black individuals, recovery from anemia ensues despite continued exposure to the offending agent. This is because the enzyme is unstable and

renders older RBCs susceptible to oxidant hemolysis while younger RBCs remain resistant. Thus, recovery from anemia occurs despite persistent exposure to the offending agent due to a high proportion of resistant reticulocytes and young RBCs with adequate enzyme levels. A similar pattern of hemolysis occurred in rats exposed to EGBE, but this effect was specific to the rat and there has been no direct evidence in the rat to link EGBE associated hemolysis to a metabolic mechanism.

1.2.1.3 Hemoglobin. The major susceptibility of the hemoglobin molecule to chemotoxicity is that of oxidant conversion to methemoglobin, with iron altered from the ferrous to the non-functional ferric state. Normally, GSH derived from the HMP shunt pathway as well as the enzyme, methemoglobin reductase protects against this conversion. Of the more than 150 hemoglobin mutants described in man, about 25 are chemically unstable and hyper-susceptible to oxidation. As is the case with G6PD deficiency, individuals with these unstable hemoglobins, develop accelerated hemolysis after oxidant exposure. Denatured hemoglobin within the affected RBCs forms aggregates which bind to, crosslink and rigidify the red cell membrane rendering the RBC susceptible to splenic destruction or phagocytic removal of the aggregates. Hemoglobin may be chemically altered by other toxins such as carbon monoxide or cyanide which render it nonfunctional.

1.2.1.4 Immune hemolytic anemia deserves special mention here because a number of drugs and toxins induce hemolysis by this mechanism. Some agents, e.g. penicillin, bind to the RBC membrane, provoke an antibody response which sensitizes RBCs to reticuloendothelial destruction. Other agents either chemically alter (e.g. alphanemethyldopa) membrane antigens or form immune complexes in the plasma which attach to RBCs as "innocent bystanders," marking them for destruction. This type of hemolytic mechanism is detected by an antiglobulin (Coomb's) test for the presence of antibody or complement on the RBC.

2.0 Evidence for hematotoxicity of EGBE. In 1943, Werner et al. first showed that EGBE as well as other monoalkyl ethers of ethylene glycol produced hemoglobinuria in mice during an acute toxicologic study. Since that time, from 1943 through 1983, more than two dozen studies have been published in eight papers, to define the hematotoxicity of EGBE. Experiments have been of acute and subchronic design and dealt with various species including mice, rats, guinea pigs, rabbits, dogs, monkeys and man. Exposures have been largely inhalational, but have also included transdermal, oral and parenteral. Dose, duration of exposures and dose-rates have varied, as have measured hematologic parameters and times of measurement. From the compilation, summary and review of these studies, which appears in Table 1, the following conclusions emerge which are explained in the discussion that follows:

1. A substantial species variation exists in susceptibility to the hemolytic effects of EGBE, with the rat most susceptible and man most resistant.
2. In the human inhalational experiment conducted, no hemolysis was demonstrated at maximal exposure up to 195ppm for 8 hours.
3. In susceptible species, the hemolytic effect of EGBE is dose-dependent with a demonstrable hemolytic threshold. Lower doses appear to induce mild alterations of RBC osmotic fragility, while maximal doses produce frank intravascular hemolysis.
4. In no species studied was there any evidence of toxicity to hematopoiesis or circulating leukocytes or platelets.

5. In susceptible rodent species, females are more prone to hemolysis than males, particularly when gravid..
6. In studies where the hemolytic sequence has been closely followed, hemolysis occurs early after initiation of EGBE exposure, is accompanied by a rise in reticulocyte count and MCV, and RBC and hemoglobin levels recover after 1-2 weeks despite continued exposure. This suggests that older RBCs are selectively hemolyzed while younger cells are resistant. (See Sec. 1.2.2)

2.1 Hemolysis and RBC alterations in rodents following exposure to EGBE. Table 1 summarizes the published toxicologic studies done to date on rodents and other species. Rodent studies include acute and subacute experiments done on mice, rats, GPs and rabbits, largely by inhalational exposures but including a few oral, transdermal and parenteral routes. In the rat, clearly the most susceptible to EGBE related hemolysis, all types of exposure produce hemolysis, indicating absorption by all routes. The well known hemolytic vulnerability of rat RBCs renders this species a particularly poor hematologic model for man. (See Appendix IV.) In the rabbit, subchronic inhalational exposures up to 200ppm produced minimal effects on RBCs while subchronic dermal applications were hemolytic at relatively high concentrations, indicating low susceptibility of rabbit RBCs to EGBE associated hemolysis. In a single subchronic study, GPs showed no hemolysis after six weeks of 5 hour daily exposure up to 494 ppm...rather striking resistance. In contrast, mice hemolyzed in acute and variable subchronic exposures up to 90 days at inhaled concentrations of EGBE of 112ppm. Thus, the susceptibility ranking among various rodent species is--rats and mice most

susceptible and rabbits and GPs least susceptible. In the studies of Carpenter, Werner, Homan and Tyl (and respective colleagues) both rats and mice exhibited crude hemolytic dose-response relationships to EGBE, as did rabbits to dermal and inhalation exposures.

2.2 Hemolysis in dogs and monkeys exposed to EGBE. The two studies of EGBE toxicity in dogs were those of Werner et al. in 1943 and Carpenter et al. in 1956. Werner exposed two dogs subchronically to inhalation of EGBE at 415ppm for 7 hours a day, 5 days per week for 12 weeks. At this relatively high concentration, a 10% decrease in hematocrit was noted at week 10 associated with a slight change in RBC osmotic fragility; no reticulocyte response was noted. Carpenter exposed 3 separate pairs of dogs to subchronic inhalational exposures of 385, 200 and 100 ppm for up to 90 days. A single female dog died 13 hours into an exposure at 617ppm and no mention is made of hemolysis in that animal. All the other dogs showed transient increase in RBC osmotic fragility, but only the dog exposed at 100ppm for 90 days developed anemia.

Carpenter's subchronic monkey EGBE inhalational experiments are difficult to interpret because the test animals had severe tuberculous infections which cause anemia. Monkeys exposed at 100ppm for 90 days showed increased RBC osmotic fragility but only transient minor decrease in red blood count. One animal exposed at 210 ppm for 30 days manifested a 50% decrease in hemoglobin by day 30, but autopsy shortly thereafter showed disseminated tuberculosis, easily capable of causing this degree of anemia.

Thus although dogs appear to be susceptible to EGBE associated hemolysis after subchronic vapor exposure, no dose-response relation- has been established. The monkey data is uninterpretable.

2.3 Relative resistance of human RBCs to EGBE associated hemolysis and alteration. The only published experiments of human inhalational exposure to EGBE are those of Carpenter, in which he exposed himself and several of his male and female colleagues to concentrations of 113ppm for 4 hours and 195ppm for 8 hours. During those exposures, ocular, mucous membrane, gastrointestinal and neurologic symptoms became the limiting toxicity; i.e. caused sufficient adverse effect that longer or greater exposure could not be tolerated. Hematologic studies showed no evidence of hemolysis or altered osmotic fragility during either exposure, even for those subjects experiencing other systemic side effects. Rats confined in the same chambers with the human subjects did hemolyze. All human subjects in both exposures excreted butoxyacetic acid in their urines indicating that they had absorbed and metabolized EGBE.

2.4 Species variation and threshold differences for EGBE associated hemolysis and red cell alterations. From the foregoing it is evident that marked species differences exist in susceptibility to EGBE related hemolysis. Clearly rats and mice are particularly susceptible and man is not. Figure 1A shows hemolytic thresholds (i.e. minimum levels of effect) for the species studied. None has been established for man, rabbits or GPs for inhalational exposures, and these three species have shown resistance to hemolysis at subchronic and acute exposure levels ranging from about 200 to 500ppm. Dogs show intermediate susceptibility and no conclusions can be drawn regarding the limited

nonhuman primate experiments. Clearly, rabbits and GPs represent far better rodent models than rats for extrapolation to man.

2.5 Putative mechanism for EGBE related hemolysis in susceptible species. The observation that in the rat, hemolysis appears selective for older RBCs invokes the "G6PD model" of a red cell age dependent enzyme or other factor. The limitation of major hemolysis induced by EGBE to mouse, rat and to lesser extent dog red cells, would tend to make one seek a factor or biochemical characteristic which selects them for EGBE induced hemolysis. EGBE from its chemical structure does not appear likely to generate oxidant radicals or induce antibody formation. Furthermore, the hemolytic pattern seen in susceptible species is inconsistent with immune hemolysis. EGBE, a lipophilic substance, might enter the lipid bilayer of the membrane and alter its fluidity or liberate lysolethacin, a hemolytic substance. Species differences in membrane lipid composition might explain differential susceptibility.

2.6 Other hematologic effects of EGBE exposure. These effects have been limited to acute stress or phase reactant effects such as elevation of WBC, particularly juvenile granulocytes, increase in fibrinogen levels, and elevated sedimentation rate. There is no evidence of alteration of bone marrow function, i.e. hematopoiesis, no abnormalities of coagulation-hemostasis or platelet function and no evidence for immunosuppression. In fact the finding that the animals are capable of a granulopoietic response after EGBE exposure is good evidence for integrity of marrow function.

3.0 Relationship of EGBE associated hemolysis with other systemic toxic effects in susceptible species. Although published studies have indicated dose-related EGBE toxicity from death to renal

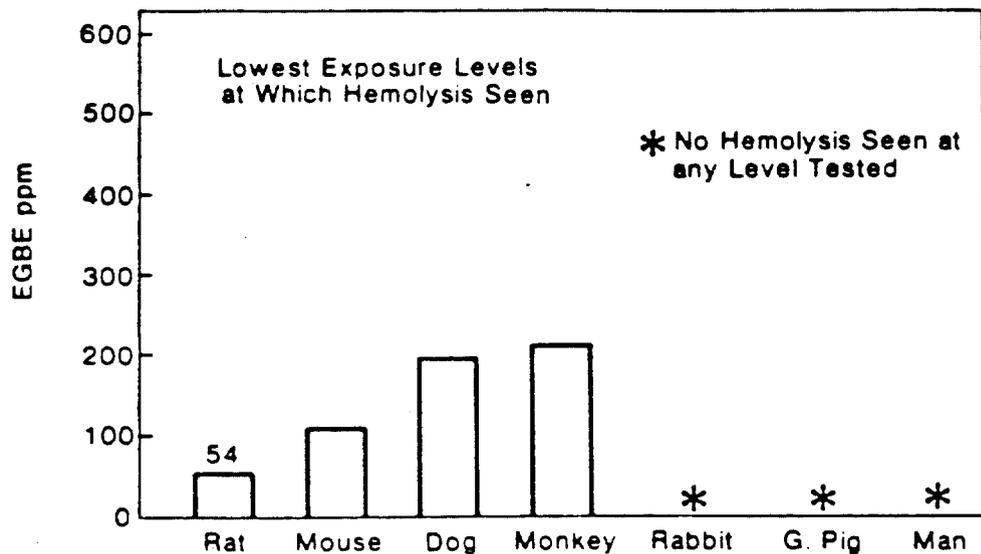
failure, at high doses to splenic follicular phagocytosis at lower doses, no definite relationship to hemolysis has been shown. The only possible exception is that of hemoglobinuric nephropathy, which was seen only in rats hemolyzing at high EGBE dose rates.

4.0 Estimation of hematologically safe EGBE exposure limits for man. EGBE has never been shown to cause hemolysis or other hematotoxicity in man. In fact, man has shown striking resistance to hemolysis following exposure to EGBE at levels clearly hemolytic for susceptible species. Even though the hemolytic mechanism of EGBE is unknown, it appears that human RBCs lack biochemical vulnerability to EGBE up to the levels tested. We do know that in 4 and 8 hour exposures to 113 and 195ppm EGBE vapors, that non-hematologic toxic effects (e.g., nasal and ocular irritation, metallic taste, headache, nausea) limited the duration and level of exposure and no blood disturbances were detected. Thus, our best estimates of subchronic effects should come from experiments involving species manifesting a hemolytic-susceptibility level similar to that of man, i.e. rabbit and GP. In those species, subchronic inhalational exposures to EGBE from 6 hours daily for 13 days at 200ppm to 7 hours daily, 5 days/wk. for 6 wks. at up to 400ppm showed no hemolysis. Other rodents, notably rats and mice represent very poor choices as animal models for hemolytic toxicity, because of their extreme susceptibility to EGBE-related hemolysis, not manifest in man. Thus, from the hematologic standpoint, a safe subchronic inhalational exposure level for man appears to exceed 200 ppm. Certainly the current ACGIH level of 25 ppm will provide an ample margin of safety.

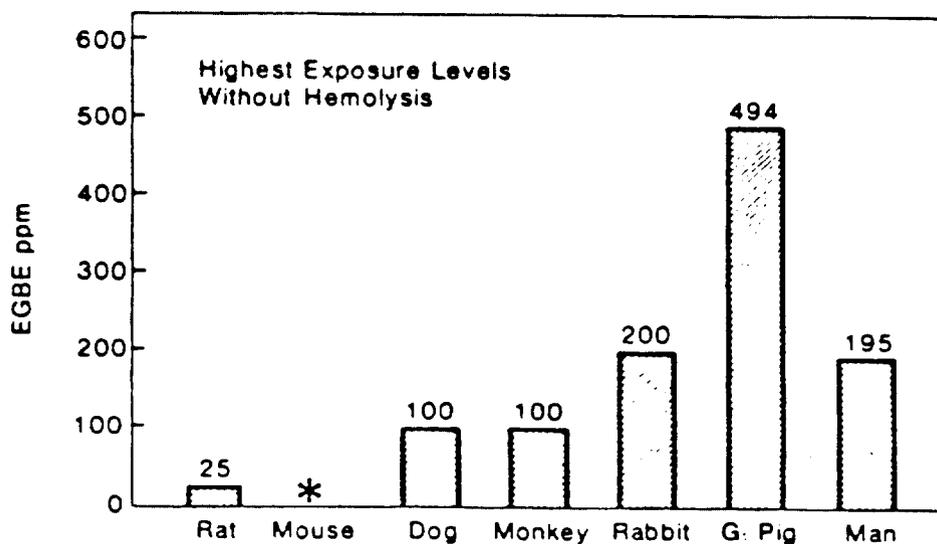
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Hemolytic Thresholds for EGBE Vapor Exposures-Species Differences



Hemolytic Resistance to EGBE Vapor Exposures-Species Differences



Relationship of EGBE Vapor Exposures to Hemolysis: Species Variation

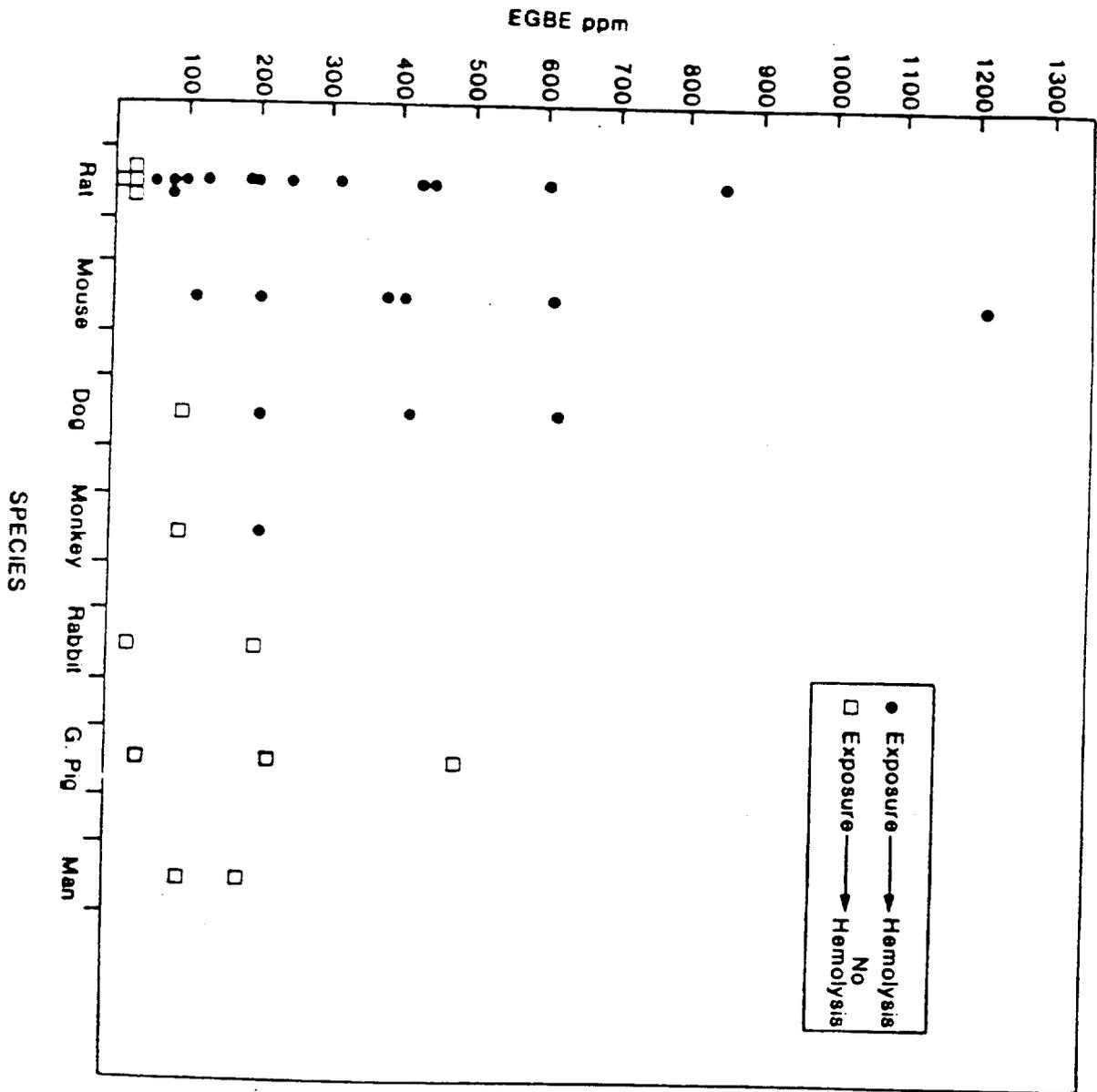


TABLE 1

SUMMARY OF PUBLISHED STUDIES OF EGBE HEMATOXICITY

EXPOSURES

AUTHOR (Ref)	YEAR	TEST ANIMAL	ROUTE	DOSE (ppm)	TIME	NO.	TYPE	HEMATOLOGIC EFFECTS	
Werner (1)	1943	Mice	I	390-1210 (range)	7 hrs	x 1	A	- hemoglobinuria at \geq 600 ppm	
					32 hrs	x 1	A	- follicular phagocytosis in spleen	
Werner (2)	1943	Rats	I	135 320	7 hrs	x 5d x 5wks	SC	- Hgb \downarrow 12.5 - 11; no retic response	
					7 hrs	x 5d x 5wks	SC	- Hgb \downarrow 12.5 - 9.5 gms 1st week with retic response; recovery at wks 4-6	
Werner (3)	1943	Dogs	I	415	7 hrs	x 5d x 12wks	SC	- elevated juvenile WBCs both groups	
								- 10% \downarrow Hct, max at wk 10	
Carpenter (4)	1956	Mice	I	112	30, 60, 90d		SC	- \downarrow RBC fragility at 30, 60, and 90 days	
								SC	- \downarrow RBC fragility, hemoglobinuria (all)
		Rats (F)	I	432	2hrs, 8hrs		A	- \downarrow RBC fragility, hemoglobinuria	
					7hrs	x 9d	SC	- in vivo hemolysis @ 2hrs, severe at 8hr	
		Rats	0	0.3-3.8 gm/kg			A	- RBC frag. \uparrow during inhalation at day 4 - 50% \downarrow RBC, 25% \downarrow Hgb	
		Rabbits	5	0.5-2 ml/kg		x 24 hrs	A	- hemoglobinuria in male at 3.0 gm/kg females at 1.5 gm/kg	
		Rats	IV						- \downarrow RBC frag; 3% \downarrow RBC
		Rats	I	54-432	7hrs	x 5d x 6wks	SC	- hemolytic at all conc. $>$ 31	
		G.P.s	I	54-494	7hrs	x 5d x 6wks	SC	- \downarrow RBC frag. at all conc; Hgburia at conc $>$ 200 ppm	
		Dogs	I	385-617	13 1/2 hrs - 28d		ASSC	- no Δ RBC frag or Hgburia	
							- Females died at 617 ppm (13%) and 6th day @ 385. Males died @ 28 days		
							- males showed \downarrow RBC frag at 7 days, returned to normal by 27 days		
							- \downarrow WBC in 385 ppm exp dogs		

TABLE 1 (continued)

EXPOSURES

AUTHOR (Ref)	YEAR	TEST ANIMAL	ROUTE	DOSE (ppm)	TIME	NO.	TYPE	HEMATOLOGIC EFFECTS
Carpenter (4) (cont'd)	1956	Dogs	I	200	31d		SC	- S1 ↑ RBC frag; ↑ MBC; S1 ↑ Hgb
		Dogs	I	100	90d		C	- WBC ↓ x 2; Hct 43 → 34; @ 90 days
		Monkeys	I	100	90d		SC	- ↓ RBC osm frag female > male
		Monkey	I	210	30d		SC	- ↓ RBC but return to normal by day 90
		Human	I	113	4 hrs		A	- ↓ RBC frag 4th day; at day 30
		Human	I	195	8 hrs		A	- Hgb and RBC ↓ by 50% (animals had TB)
								- no Δ in RBC fragility, Hgb
								- no Δ in RBC fragility, Hgb
								- limited by non-hematol Sx
								- females less tolerant than males
								- human subjects excreted butoxy-acetic acid
Homan (5)	1979	Rabbits	S	2% - 100%	6 hrs	x 9d	SC	- 100% EGBE caused hemoglobinuria
								↓ Hct, ↑ RBC frag. by day 2
								- 5 females with Hgburia at day 9 @ 50%
								- ↓ RBC, Hgb in females at day 9 @ 50%
								- no hematol. effects @ 25% or less
Wil Res (6) Labs	1982	Rabbits	S	2.8% - 42.8% (10-100 mg/kg/day)	6 hrs	x 5d x 13 wks	SC	- males ↑ RBC @ 14% at 4 wks (?)
								- females ↑ RBC @ 14% at 4 wks
								- RBC osm. frag. nl. at 12 wks
Tyl (7)	1983	Rabbits	I	25 - 200	6 hrs	x 13d	T	- no change in RBC, Hgb, Hct, osm. frag. or indices
		Rats	I	25 - 200	6 hrs	x 9d	T	- ↓ RBC at 100 and 200 ppm
								- hemoglobinuria
Dodd (8)	1983	Rats	I	200 - 850	4 hrs	x 5d	A	- LC50 450; hemoglobinuria
			I	25 - 250	6 hrs	x 9d	SC	- ↑ RBC, Hgb, ↑ Retic. at 86 + 245 ppm
			I	10 - 75	6 hrs	x 5d x 13 wks	SC	- values returned to nl. by day 14
								- 5% ↑ RBC and Hgb, ↑ HCH only at 77 ppm

Abbreviations: I = Inhalation
S = skin
O = oral
IV = intravenous

A = acute
SC = subchronic
C = chronic
T = teratology

APPENDIX I

Histophysiology of hematopoiesis. In mammalian species the marrow spaces of cancellous bone is the principle anatomic site of blood cell formation. In the human fetus and lower mammals such as rodents, the liver and spleen are also hematopoietically active. However, in adult man, blood cell production is confined to the bone marrow of the axial skeleton. Within this site, marrow function requires an hematopoietic inductive microenvironment with its precise biochemical balance of cations, pH, pO₂, mucopolysaccharides and protein transport substances, hormones and growth factors. This critical microenvironment is particularly susceptible to disruption by offending chemical agents since its inherent capacity for detoxification is limited. Structurally, this marrow ground substance surrounds a reticular fibrous network laid down by large interstitial reticular cells, found within marrow spaces. Circulating blood perfuses the marrow through sinusoids lined by endothelium and surrounded by adventitial cells which control access of newly formed erythrocytes, leukocytes and platelets to the peripheral blood. Damage to this "marrow-blood barrier" is evidenced by the abnormal presence of immature red and white cell precursors in the blood. Within the marrow spaces, erythropoietic islands are located close to sinusoids, megakaryocytes--producing platelets--are tightly juxtaposed to sinusoidal margins and granulopoietic elements are more distant from sinusoids since their progeny, mature granulocytes are capable of active locomotion into the sinus lumen.

APPENDIX II.

Mechanisms and control of hematopoietic cell proliferation.

Hematopoiesis begins with a multipotential stem cell which maintains the capacity for continuous self-renewal and differentiates into "committed" stem cells, specific for each of the three hematopoietic cell lines--erythropoiesis, granulopoiesis and thrombopoiesis--as well as precursors for immunocytes, the T- and B-lymphocytes and plasma cells responsible for cellular and humoral immune function. Each of these cell lines is under a specific system of humoral feedback regulation which responds to signals from the periphery and controls the rates of production and release of their end products, the erythrocytes, leukocytes and platelets.

A. Erythropoiesis. Committed erythropoietic stem cells (referred to as BFU-e and CFU-e for burst and colony forming units) initiate erythropoiesis under stimulus from the hormone, erythropoietin. The anatomic unit of erythropoiesis is the erythropoietic island, composed of a central reticular cell surrounded by a corona of developing erythroblasts which mature from proerythroblast to orthochromatic erythroblast. During the average maturation time of 72 hours, erythroblasts undergo a series of four maturational cell divisions which produces amplification such that a single proerythroblast eventually yields a total of sixteen mature red blood cells (RBCs.) The critical biochemical events for this

process include DNA synthesis, for nuclear replication, synthesis of hemoglobin, membrane-proteins and enzymes, as well as membrane lipid synthesis. As the orthochromatic erythroblast leaves the erythroblastic island it actively ejects its nucleus to become a reticulocyte, a young RBC held in reserve in the marrow for 24-48 hours until its release into the blood in response to a peripheral stimulus. The servomechanism which controls both erythropoietic rate and reticulocyte release is dependent upon oxygen sensors in the kidney which secrete erythropoietin in response to a fall in tissue oxygen tension. Erythropoietin activates the erythropoietic stem cell, and accelerates maturation and amplification of erythroblasts while simultaneously releasing stored reticulocytes into the blood. Under conditions of erythropoietic stress such as acute blood loss, hemolysis, or hypoxia, the maturation-amplification process may be accelerated 2-3 times with a potential six-fold increase in total erythropoiesis.

B. Granulopoiesis. The formation of the granulated leukocytes of the blood occurs in the marrow by a process of maturation-amplification similar to that of the erythroid series. Because of the much more rapid turnover of peripheral blood granulocytes, marrow granulopoiesis exceeds erythropoiesis by a ratio of 5 to 1. The earliest granulopoietic element is the committed stem cell or CFU-g/m (for granulocyte-monocyte colony forming unit.) By a series of maturational divisions and amplification steps, granulopoiesis is capable of rapidly expanding its proliferating pool and accelerating its production rate greater than 20-fold, in response to increased

peripheral demand as with acute bacterial infection or trauma. In contrast to erythropoiesis, granulopoiesis is under control of a positive feedback control loop, such that substances released from active mature granulocytes in blood and tissues accelerate the granulopoietic rate, principally by stimulating cell replication at the myelocyte stage.

C. Thrombopoiesis. Platelets are produced by megakaryocytes, large multinucleated marrow cells located adjacent to sinusoids. Stimulated by the hormone, thrombopoietin, megakaryocytes (derived from CFU-M) manifest nuclear division without cell division so that progressively large multiploid cells are produced. These cells produce platelet constituents within their cytoplasm and bud thousands of platelets from their periphery for direct release into the circulating blood. The platelets, essential for control of hemorrhage and blood vessel repair, release a variety of factors when actively consumed in these processes. One such factor, platelet-derived-growth-factor (PDGF) stimulates blood vessel repair by activating endothelial cell proliferation. Another, platelet factor-3 activates the coagulation process. When platelets are rapidly consumed or destroyed in the periphery, thrombopoietin, released by endothelial cells, accelerates thrombopoiesis.

D. Immunocytes. The immunologic effector cells, the thymus-derived (T-) lymphocytes, bone marrow-derived (B-) lymphocytes and plasma cells are also produced from the multipotential hemapoietic

stem cell and partially reside in the marrow. These cells form part of the larger family of immunocytes which function within the thymus gland, spleen, lymph nodes and marrow, and circulate through the blood and lymphatic vessels. T-cells of several types are responsible for cellular immune function and immunologic "memory"; B-lymphocytes and bone marrow plasma cells produce antibodies, which sensitize bacteria and other foreign "invaders" for destruction by granulocytes and histiocytic cells. In general, any agent, chemical or physical, which is toxic to hematopoiesis, is also immunosuppressive. Thus, the immune system is mentioned here, but will not be given further detailed consideration in this discussion since there is no evidence that EGBE has any immunosuppressive effect.

APPENDIX III. Peripheral blood cells as targets for chemotoxicity.

A. Peripheral blood granulocytes (WBCs). Chemotoxic effects on WBCs are less well understood than on RBCs. WBCs must be capable of motility, chemotaxis, phagocytosis, and bacterial killing and digestion. To carry out these functions, they must have an intact actinomyosin fibrillar system, ample supply of ATP, adequate GSH to neutralize excessive oxidant radicals and sufficient bactericidal enzyme activity. Such functions obviously have many of the same biochemical requirements and therefore vulnerabilities as RBCs. G6PD deficient WBCs show impaired phagocytosis and killing particularly in the face of oxidant chemicals. Cyanide and fluoride poison the respiratory burst of bactericidal activity, preventing killing. Anesthetic agents and surface active compounds as well as lipophilic agents (e.g.

diethylether) impair chemotaxis and phagocytosis. Thus, WBCs are most likely equally susceptible to chemotoxicity as RBCs, but the difficulties in detection of even major changes in function make WBC function an unlikely subject for routine toxicologic study.

B. Lymphocytes. T- and B-lymphocyte functions, cellular and humoral immunity respectively, may be both stimulated and depressed by chemical agents. The presence and function of these lymphocytes and their various subsets are easily detected and analysed by modern immunologic methods employing monoclonal antibodies and flow cytometry among others. The proliferative response of both T- and B-lymphocytes to specific plant lectins and their elaboration of certain lymphokine substances is standard in most immunology labs. Thus, even though toxic chemical immunosuppression and stimulation may be easily (though expensively) measured, they are rarely the subject of environmental toxicologic study.

C. Platelets (TBCs). These small anuclear cells are easy to quantitate in the peripheral blood and their aggregation function and release reaction essential to normal hemostasis and vascular repair in the host is easily measured in the standard laboratory aggregometer. Platelets are quite sensitive to chemical injury, particularly with respect to their critical prostaglandin metabolism. This requires the integrity of several enzymatic steps for conversion of arachadonic acid to cyclic prostaglandin intermediates and thromboxane-A₂ (TXA-₂) essential for platelet aggregation. The cyclo-oxygenase step is most vulnerable to inhibition by a variety

of compounds including aspirin and related antiinflammatory agents. Aromatic compounds found in garlic, mushroom and other foods and toxins inhibit this enzyme as well. The phosphodiesterase step, which liberates arachidonate from phospholipids is also sensitive to chemical inhibition. Unfortunately, the quantitative evaluation of platelets employed in most toxicologic studies, provides no information concerning their function.

APPENDIX IV: HEMOLYTIC SUSCEPTIBILITY OF THE RAT RED BLOOD CELL

The circulatory survival of the red blood cell (RBC) in all mammalian species is dependent upon the metabolic integrity of the cell, the elasticity of its membrane and the fluidity of its intracellular hemoglobin. These factors are determinants of the deformability of the RBC, a property essential to the ability of the cell to circulate through tight passages of the spleen and microcirculation. The RBC of the rat has known since the 1930's to differ in these characteristics from that of man and other mammalian species. In his classic monograph, Hemolysis and Related Phenomena[1], Eric Ponder focuses upon the "paracrystalline state of the rat red cell", noting the "extreme case of anomalous osmotic behavior" that the rat RBC presents. Drabkin, in 1945, described the rat RBC as containing "hemoglobin in an unusually metastable state approaching that of incipient crystallization[2]." In an electron microscopical study utilizing the freeze-etching method, Lessin demonstrated the molecular structure of crystals within the rat red cell, providing direct evidence of the lack of fluidity of the hemoglobin within the rat RBC[3]. In a later paper, Lessin showed the similarity between the rat RBC and that of human homozygous hemoglobin C disease, an inherited hemolytic anemia of man in which the dense paracrystalline state of the hemoglobin leads to premature destruction of the RBC[4]. Taken together, these observations indicate that the rat RBC contains densely packed paracrystalline hemoglobin which decreases the deformability of the RBC and renders it more susceptible to hemolysis.

Rat hemoglobin (contained in two distinct major electrophoretic bands) is less chemically stable than human hemoglobin A and more susceptible to oxidant hemolysis. Because the hexosemonophosphate shunt pathway of the rat RBC is less active than that of the normal human RBC, the rat cell can not as effectively generate reduced glutathione, to protect the hemoglobin and membrane structural proteins against oxidant damage. The concomitant chemical alteration of hemoglobin and the cytoskeletal proteins of the membrane promotes chemical crosslinkage between altered hemoglobin and membrane decreases membrane fluidity of the rat RBC.

As pointed out by Ponder, the rat red cell manifests "extreme anomalous osmotic behavior." [1]. This indicates that the rat RBC, while smaller than that of man has a higher corpuscular hemoglobin concentration, is more dense, more spherical and has a lower surface area to volume ratio. By definition, this renders the rat RBC more susceptible to osmotic lysis than that of man.

Taken together, these observations concerning the rat RBC provide substantial evidence for its innate hemolytic susceptibility and its particular vulnerability to chemical insult from compounds such as EGBE. The rat, therefore, represents an especially poor toxicologic animal model for the evaluation of the hemolytic potential of EGBE in man.

References:

1. Ponder, E., Hemolysis and Related Phenomena, Grune & Stratton, New York, 1948, pp. 96-101.
2. Drabkin, D. Science 101: 445-7, 1945.
3. Lessin, L. Nouv. Rev. Fr. d'Hemat. 8: 423-435, 1968.
4. Lessin, L., et al., J. Exp. Med. 130: 443-466, 1969.

APPENDIX V

LABORATORY DETECTION OF HEMOLYSIS OR ERYTHROCYTE ALTERATION

Because hemolytic anemias comprise 20% of all anemias in man, clinical diagnostic hematology laboratories have developed testing procedures to demonstrate both the presence and mechanisms of most clinical hemolytic states. These studies may be divided as follows:

Screening tests for hemolysis-

- decreased hematocrit (RBC or Hgb.)
- microscopic exam of RBCs for cell shape abnormalities
- reticulocyte count >2% (corrected)
- elevated serum indirect bilirubin
- elevated urinary urobilinogen
- decreased serum haptoglobin
- presence of urinary hemosiderin
- elevated serum lactic dehydrogenase

Proof of hemolysis-

- presence of free plasma hemoglobin
- shortened RBC circulatory survival (by isotopic, carbon monoxide generation, or Ashby methods)

Mechanism of hemolysis

- hemoglobin electrophoresis
- hemoglobin gene mapping
- RBC enzyme activity measurements and electrophoretic identification
- RBC membrane fragility (fragillograph, ektacytometer)
- RBC membrane lipid and protein analyses
- Identification of antibodies to RBCs in plasma or on cells; complement components on RBC surface.

In most clinical cases, selected screening tests are sufficient to establish presence of hemolysis, such that "proofs" are unnecessary. One then usually proceeds to determine the mechanism of hemolytic anemia, since that will lead to the precise diagnosis and indicate therapy.

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March 19, 1986

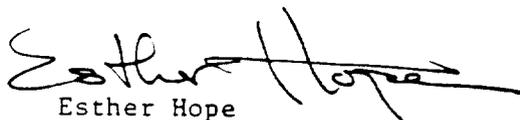
Dr. Ralph Gingell
Shell Development Comp
One Shell Plaza
P. O. Box 4320
Houston, Texas 77210

Dear Dr. Gingell:

Enclosed is the information you requested on fertility assessment of ethylene glycol monobutyl ether. A copy of the full report can be obtained from the National Technical Information Services, 5285 Port Royal Road, Springfield, VA 22161 (NTIS Accession #PB85226827/AS).

If I can be of further assistance, please let me know.

Sincerely,


Esther Hope
Laboratory Manager

EH/lf

Enclosure

2514 Regency Road, Suite 105, Lexington, KY 40503
Phone 606-278-0603

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Abstract

Ethylene glycol monobutyl ether (butyl cellosolve, 2-butoxyethanol, butyl oxitol, EGMBE) is a colorless mobile liquid with a slightly rancid odor, an initially bitter taste followed by a burning sensation, and numbness of the tongue. EGMBE is readily absorbed through the skin.

Industrial uses of EGMBE include:

1. chiefly in the lacquer industry where its advantage results from its high boiling point and slow hardening which gives a slight increase of gloss to the film;
2. in cleaning materials for metals and dry cleaning soaps;
3. in the textile industry for dyeing and printing;
4. in hydraulic fluids.

Werner and his associates (1943) compared the relative toxicity of EGMBE and a series of other glycol ethers. The minimal lethal concentration of EGMBE after a single 7-hour exposure of mice was 700 ppm. Furthermore, hemoglobinuria was common at concentrations near the lethal levels.

Dyspnea was the most common sign of intoxication. Notable changes were seen in the lung, kidney, as well as in the liver.

Carpenter et al. (1956) exposed mice, rats, rabbits, guinea pigs, dogs, and monkeys to EGMBE vapors and observed the effect of hemolytic anemia and its sequelae. While rodents did not tolerate repeated exposure at 200 ppm this

level was only slightly injurious to dogs. These authors suggested, on the basis of industrial experience and volunteer short-term exposures, that humans were relatively less susceptible than experimental animals to EGMBE. In several single, 8-hour exposures of humans at levels of 100 and 200 ppm, no objective effects were seen except for urinary excretion of butoxyacetic acid.

Nagano et al. (1979) orally dosed male mice once daily with EGMBE 5 days/week and for 5 weeks. EGMBE dosing had no significant effect on testicular weight, both absolute and relative to body weight.

Nelson et al. (1979) exposed pregnant rats on days 7 to 15 of gestation to a series of EGMBE levels. At up to 200 ppm, EGMBE was neither teratogenic nor embryotoxic.

The reproductive toxicity of EGMBE was evaluated according to the Fertility Assessment by Continuous Breeding (FACB) protocol (Appendix I). CD-1 mice, purchased from Charles River Breeding Laboratory, Inc. (Kingston, NY), were utilized. During quarantine, experimental animals were checked for endoparasites and antibodies for 11 known mouse viruses. The first batch of animals was positive for the sendai virus antibodies. These animals were designated unacceptable for the Task 2 study. The vendor replaced the animals.

EGMBE was obtained from Chem Central via Midwest Research Institute (MRI), Kansas City, MO (MRI assigned lot #C092882). MRI (under the contract to NTP) checked the purity and stability of the chemical. A copy of the MRI report is attached (Appendix II). EGMBE was administered in drinking water available ad libitum. The treatment solutions were formulated by mixing the test article (weight/volume) directly into different proportions of distilled water. All dosing solutions were prepared every two weeks or more often if necessary. Aliquots of representative dosage formulations were submitted to MRI for chemical analysis. Reference aliquots were within 97 to 104 percent of the intended EGMBE concentrations (Table 1). These limits were considered acceptable by the Project Officer. The detailed reports describing analysis of various reference samples are attached (Appendix III).

In Task 1, the dose-range finding study, EGMBE was tested at the following concentrations: 0.0 (control), 0.25, 0.5, 1.0, 2.5, and 5.0% (w/v). During the 2 weeks of Task 1, the untreated males, on the average, gained 7% of their original body weight. The male mice exposed to 5.0% EGMBE, however, lost 31% of their initial body weight (Table 2 and Table A1 of the Supplementary Data). The female CD-1 mice responded similarly to EGMBE treatment. Two out of the 8 male mice died in the 5.0% dose group. Five out of the 8 female mice died in the 5.0% dose group. No male or female

mice died in the control or any of the remaining dose groups (Table 2 and Table A1 of the Supplementary Data). A distinct dose-related decrease in daily water consumption values was also observed (Table 2 and Table A2 of the Supplementary Data).

Task 2 was the continuous breeding phase of the protocol. This phase employed a vehicle control group (40 males and 40 females) and three dose groups (20 males and 20 females per group). Since it was required that the highest dose should not suppress body weight gain more than 10 percent compared to controls and allow 90 percent or greater survival in male and female CD-1 mice, 2.0% EGMBE was chosen for the high dose. The mid-dose was 1.0% and the low dose, which was expected to be a no effect dose level, was 0.5%. The route of administration was once again drinking water. Eleven-week old male and female CD-1 mice were exposed to the chemical during a 7-day pre-mating period, after which they were randomly paired (one male: one female) within each dose group and cohabited for 14 weeks. New born litters were evaluated and immediately sacrificed.

EGMBE was significantly toxic to female CD-1 mice; during Task 2 cohabitation 13 out of the 20 females died in the 2.0% dose group as compared to 1 each in the control and 0.5% groups and 6 in the 1.0% group (Table 3). Male mice in the control and 0.5% EGMBE groups gained an average of 5 and 3% of their original body weights, respectively, after 14

weeks of treatment (Table A3 of the Supplementary Data). On the contrary, male mice in the 1.0 and 2.0% dose groups lost approximately 1 and 2% of their initial body weights, respectively. Group mean body weights for the females varied with the gestation phase except for animals in the 2.0% dose group; the average body weight values were consistently lower than the corresponding control values.

Daily consumption of dosed water by animals in the three treatment groups was consistently lower than the control value and the response was dose-related (Table A4 of the Supplementary Data). This response may be related to the bitter taste of EGMBE and its ability to cause a burning sensation. The average water consumption and mean body weight data indicated that the male mice in the 0.5, 1.0, and 2.0% dose groups received approximately 0.724, 1.345, and 2.044 g/kg bw of EGMBE, respectively.

All breeding pairs in the 0.5% dose group were fertile (delivered at least one litter). In the control and 1.0% dose group, all except 1 pair each were fertile (Table 3). In the high dose group (2.0% EGMBE) 5 out of the 7 breeding pairs were fertile. It must be emphasized that data from pairs in which one or both partners died during the cohabitation phase were excluded for intergroup comparisons and statistical analysis.

There was a significant decrease ($p < 0.05$) in the mean number of litters per fertile pair at the 1.0 and 2.0% EGMBE levels (Table 4). Other reproductive parameters significantly affected at these dose levels were: the number of live pups (male, female, as well as combined) per litter ($p < 0.01$); the proportion of pups born alive ($p < 0.01$); and the live pup weights (both absolute and adjusted; $p < 0.05$). The live pup weight values were also significantly affected ($p < 0.01$) in the 0.5% dose group.

The continuous breeding portion of the protocol, Task 2, indicated that EGMBE treatment significantly affected fertility both at the 1.0 and 2.0% dose levels. The observed response, however, can be attributed to severe toxicity at these dose levels. Since Task 2 does not discriminate which sex (or sexes) is susceptible to the chemical exposure it was interfaced with a crossover mating study, Task 3. In this particular study, although not required by the protocol, reproductive performance of the last and generally the fifth litter (second generation) from the control and 0.5% dose groups was evaluated (Task 4). It must be added that it was not feasible to conduct Task 4 using second generation mice from the 1.0% dose group because only 5 breeding pairs delivered live pups during the holding period.

During Task 3, animals from the 1.0% dose group were tested in a crossover mating trial to determine whether the males or females or both sexes had compromised reproductive performance when matched with control animals. The females which were treated with 1.0% EGMBE and cohabited with control males had 46% fertile matings as compared to a control value of 79% (Table 5). The corresponding value for 1.0% male X control female group was 75% (Table 5). There was also a decrease with respect to the number of live pups per litter and the proportion of pups born alive but the difference was not statistically significant ($p > 0.05$). The average live pup weight (absolute and adjusted) in the control male X 1.0% female group was significantly affected ($p < 0.05$; Table 6). These parameters were not affected for pups delivered by control females mated with EGMBE treated males (Table 6).

At the end of Task 3, both male and female mice were necropsied. Detailed sperm morphology and vaginal cytology evaluations were also performed (Appendix IV).

At necropsy, group mean body weight of treated female mice was significantly lower ($p < 0.01$) than the control group, but the average kidneys weight was higher ($p < 0.01$). There was no difference ($p > 0.05$) with respect to the average liver weight (Table 7). The organ weights were adjusted for body weight at necropsy. The mean adjusted kidney and liver weights in treated animals were significantly higher ($p < 0.01$)

than the corresponding control values (Table A10 of the Supplementary Data). The body and organ weights for individual female mice are presented in Table A11 of the Supplementary Data.

For male mice, no significant differences ($p > 0.05$) were noted with respect to the average liver, right cauda, right epididymis, prostate, kidneys, and seminal vesicle(s) weights at necropsy (Table 8) between the control and treated mice. The whole body and liver weights were significantly affected ($p < 0.05$). Male organ weights were also adjusted for body weight at necropsy (Table A12 of the Supplementary Data); the average kidney weights were significantly higher for treated mice ($p < 0.05$) than the control value.

The cauda epididymal sperm counts were essentially identical for control and treated male mice (Table 9 and Appendix IV). The sperm motility values for the control and treated male mice were 93 and 94%, respectively (Table 9). The incidence of abnormal sperm was also not affected ($p > 0.05$) by 1.0% EGMBE treatment (3.5% vs. 4.1%, Table 9). EGMBE treatment did not appear to interfere with the relative frequency of various estrous stages or the average estrous cycle length (Table 10).

The results of the crossover mating trial (Task 3) indicate that the reproductive capacity of female mice is relatively more susceptible to the effects of EGMBE than the

males under the same exposure conditions. Interestingly, the average kidney weights in both male and female CD-1 mice drinking water containing EGMBE was significantly higher ($p < 0.05$) than the control group. These observations prompted a detailed histopathologic evaluation of kidneys from control and treated female CD-1 mice. No treatment related lesions were identified in the kidneys of the female CD-1 mice exposed to EGMBE. The results from the pathology studies are described in detail in Appendix V.

As previously described, Task 4 is designed to evaluate the reproductive performance in the offspring from the final Task 2 litters of the control and high dose groups. In this particular study, pups delivered by the control and low dose (0.5% EGMBE) groups were utilized. Pups were weaned 3 weeks after delivery. Pups continued to receive the chemical treatment initially through lactation until weaning and later through drinking water. Animals were mated at 74 ± 10 days of age. Twenty second generation male mice were randomly mated with 20 second generation female mice both in the control and 0.5% EGMBE groups. Mating was continued until a copulatory plug was found or for 7 days, whichever was less.

Second generation animals were weighed at weaning, the first day of cohabitation, and once a week thereafter. Daily water consumption was also monitored once a week beginning with the week of cohabitation. These data are illustrated in Tables A14 and A15 of the Supplementary Data.

The mating index (percent of plug positive/no. cohabited) for both the control and treated second generation animals was 85% (Table 11). The fertility index (percent of no. fertile/no. cohabited) for the control and treated pairs was 80 and 75%, respectively (Table 11). No significant differences ($p > 0.05$) were noted with respect to the other reproductive parameters such as litter size, proportion of pups born alive, and sex ratio. The reproductive performance of second generation individual breeding pairs is presented in Table A16 of the Supplementary Data.

At the end of Task 4, all second generation animals were necropsied. The reproductive tract and gonadal tissues were weighed. Second generation female mice body and organ weights are summarized in Table 13. Group mean kidneys weight at necropsy for animals exposed to 0.5% EGMBE was significantly higher ($p < 0.05$). No apparent differences existed with respect to the average body and liver weights. Organ weights were adjusted for body weight at necropsy. The group mean weights for kidneys and liver were significantly different ($p < 0.05$) from the control value (Table A17 of the Supplementary Data). The individual body and organ weights for second generation female mice are presented in Table A18 of the Supplementary Data.

The average body weight at necropsy for treated second generation male mice was 36.9 g. The corresponding value

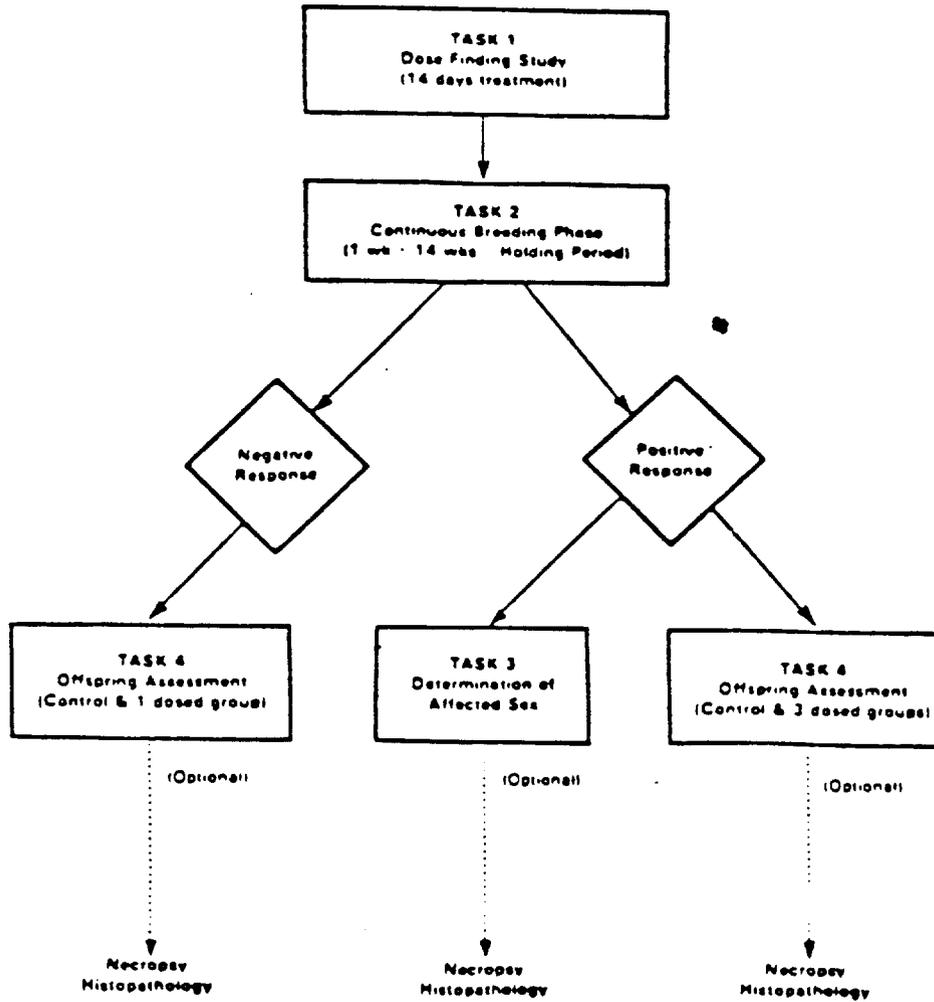
control male mice was 37.0 g. The group mean organ weights for the liver, right testis, right cauda, right epididymis, prostate, seminal vesicles, and kidneys were also not significantly ($p > 0.05$) different than the control group (Table 14). Organ weights were adjusted for body weight at necropsy (Table A19 of the Supplementary Data). Adjusted organ weights for the control and the treated group were essentially similar ($p > 0.05$), except for the liver ($p < 0.01$).

Sperm morphology and vaginal cytology studies were also conducted when second generation animals were necropsied (Appendix IV). Briefly, EGMBE treatment at the 0.5% dose level had no significant effect on the incidence of abnormal sperm, average sperm motility, and sperm density (Table 15). Vaginal smears were prepared for 7 days prior to necropsy from all second generation female mice. The average estrous cycle length in the control and treated animals was 4.4 and 5.1 days, respectively (Table 16). No significant differences were noted with respect to the relative frequency of different estrous stages (Table 16).

In essence, EGMBE administered in drinking water caused significant mortality especially at the 1.0 and 2.0% dose levels. EGMBE treatment was relatively more toxic to females; the female partner from 6 and 13 breeding pairs in the 1.0 and 2.0% dose groups, respectively, died during Task

2 cohabitation. Fertility and reproductive performance was significantly affected by EGMBE treatment only at the toxic dose levels (1.0 and 2.0%). Reproductive parameters affected during Task 2 were: (1) the number of litters per pair (2) the number of live pups per litter; (3) the proportion of pups born alive; and (4) both absolute and adjusted live pup weights. From the Task 3 results it was apparent that fertility in female CD-1 mice is relatively more sensitive to EGMBE treatment than the male mice. EGMBE treatment at the 0.5% dose level did not significantly affect the fertility and reproductive performance of either first or second generation CD-1 mice.

Figure 1: FERTILITY ASSESSMENT BY CONTINUOUS BREEDING
FLOW DIAGRAM *



* This is a revised version of the flow diagram. According to the original flow diagram, if the overall response during Task 2 was positive, only Task 3 was conducted.

Table 2. Summary of Body Weight and Daily Water Consumption Data (Task 1) Ethylene Glycol Monobutyl Ether

Treatment Group	Number of Animals	Percent Mortality d(0-14)	Body Weight (g)		Percent Change In Body Weight d(0-14)	Daily Water Consumption (g)		Daily Dose (g/kg bw) ^b
			d-0	d-14		d-0 to 7	d-7 to 14 (g/kg bw)	
Control (0.0 mg/mL)	M	0	35.2 ± 0.92	37.5 ± 0.88	+7	6.85 ± 0.12	5.92 ± 0.56	0.0
	F	0	26.0 ± 0.45	29.0 ± 0.40	+12	6.85 ± 0.12	7.02 ± 0.12	0.0
0.250 (2.5 mg/mL)	M	0	36.4 ± 0.77	39.6 ± 0.87	+9	6.51 ± 0.31	6.41 ± 0.31	0.43
	F	0	27.5 ± 0.29	30.4 ± 0.57	+11	5.55 ± 0.31	5.70 ± 0.34	0.50
0.50 (5.0 mg/mL)	M	0	35.7 ± 0.67	37.0 ± 0.81	+6	5.28 ± 0.21	5.36 ± 0.29	0.73
	F	0	25.9 ± 0.56	28.1 ± 0.53	+8	3.96 ± 0.29	4.00 ± 0.26	0.75
1.00 (10.0 mg/mL)	M	0	35.7 ± 0.49	37.3 ± 0.70	+5	4.06 ± 0.23	4.02 ± 0.31	1.13
	F	0	25.6 ± 0.31	26.7 ± 0.59	+4	3.15 ± 0.12	2.99 ± 0.12	1.20
2.50 (25.0 mg/mL)	M	0	36.0 ± 0.45	32.8 ± 1.7	-4	2.27 ± 0.09	2.78 ± 0.27	2.05
	F	0	25.7 ± 0.42	23.4 ± 1.54	-4	1.74 ± 0.13	2.21 ± 0.22	2.38
5.00 (50.0 mg/mL)	M	0	35.4 ± 0.45	24.3 ± 1.42	-31	1.97 ± 0.12	2.23 ± 0.24	4.20
	F	0	26.5 ± 0.51	18.1 ± 1.42	-32	1.79 ± 0.13	1.97 ± 0.26	5.11

a: D = 0 represents the first day of treatment, and d = 14 represents the last day of treatment.
 b: Daily dose is based on average body weight on day 7.

b: The female partner died during cohabitation in 1, 1, 6, and 13 breeding pairs from the control, 0.5, 1.0, and 2.0% dose groups, respectively; data from these breeding pairs were excluded.

a: Fertility Index (%) = $\frac{\text{No. Fertile}}{\text{No. Cohabited}} \times 100$

Treatment Group	No. Fertile/ No. Cohabited	Fertility Index (%) ^a
Control	38/39 ^b	97
0.5%	19/19 ^b	100
1.0%	13/14 ^b	93
2.0%	5/7 ^b	71

Table 3. Fertility of Pairs During Continuous Breeding (Task 2) Ethylene Glycol Monobutyl Ether

Table 4. Reproductive Performance of Fertile Pairs During Continuous Breeding (Task 2) Ethylene Glycol Monobutyl Ether

Reproductive Parameter	Treatment Group		
	Control	0.5%	1.0%
LITERS PER PAIR	4.68 ± 0.126(30) ^b	4.95 ± 0.053(19)	3.69 ± 0.414(13) ^c
LIVE PUPS PER LITTER			
Male	5.56 ± 0.249(30)	4.81 ± 0.280(19) ^e	2.75 ± 0.466(13) ^d
Female	5.74 ± 0.254(30)	5.14 ± 0.302(19)	2.71 ± 0.475(13) ^d
Combined	11.30 ± 0.430(30)	9.96 ± 0.473(19)	5.47 ± 0.803(13) ^d
PROPORTION OF PUPS BORN ALIVE	0.97 ± 0.016(30)	0.96 ± 0.023(19)	0.61 ± 0.088(13) ^d
SEX OF PUPS BORN ALIVE (MALES/TOTAL)	0.49 ± 0.013(30)	0.40 ± 0.018(19)	0.50 ± 0.043(13)
LIVE PUP WEIGHT (g)			
Male	1.64 ± 0.013(30)	1.50 ± 0.020(19) ^e	1.53 ± 0.032(13) ^g
Female	1.50 ± 0.012(30)	1.53 ± 0.019(19) ^e	1.45 ± 0.029(13) ^c
Combined	1.61 ± 0.013(30)	1.56 ± 0.019(19) ^e	1.49 ± 0.030(13) ^c
ADJUSTED LIVE PUP WEIGHT (g) ^h			
Male	1.65 ± 0.015(30)	1.50 ± 0.020(19) ^g	1.51 ± 0.025(13) ^c
Female	1.60 ± 0.014(30)	1.53 ± 0.018(19) ^g	1.42 ± 0.023(13) ^d
Combined	1.63 ± 0.014(30)	1.56 ± 0.019(19) ^g	1.46 ± 0.024(13) ^d

a: Mean ± SE.
 b: Number of fertile pairs providing the data indicated in parentheses.
 c: Significantly different (p<0.05) from the control and 0.5% groups.
 d: Significantly different (p<0.01) from the control and 0.5% groups.
 e: Significantly different (p<0.05) from the control group.
 f: No live pups in one litter.
 g: Significantly different (p<0.01) from the control group.
 h: Means adjusted for total number of live and dead pups per litter by analysis of covariance.

c: Although not detected by direct means, 4 females were scored plug-positive based on the delivery of a litter.
 d: Although not detected by direct means, 1 female was scored plug-positive based on the delivery of a litter.

a: Mating Index (%) = $\frac{\text{No. with Copulatory Plugs}}{\text{No. Cohabited}} \times 100$
 b: Fertility Index (%) = $\frac{\text{No. Fertile}}{\text{No. Cohabited}} \times 100$

Treatment Group	No. with Copulatory Plugs/ Mating Index (%) ^a	No. Fertile/ No. Cohabited	Fertility Index (%) ^b
Control Male X Control Female	16/19c	84	79
Control Male X 1.0% Female	11/13d	85	48
Control Male X Control Female	17/20d	85	75

Table 5. Mating and Fertility of Pairs After a Mating Trial to Determine the Affected Sex (Task 3) Ethylene Glycol Monobutyl Ether

Table 6. Reproductive Performance of Fertile Pairs After a Mating Trial to Determine the Affected Sex (Task 3) Ethylene Glycol Monobutyl Ether

Reproductive Parameter ^a	Treatment Group		
	Control Male X Control Female	Control Male X 1.0% Female	1.0% Male X Control Female
LIVE PUPS PER LITTER			
Male	4.27 ± 0.733(15) ^b	2.83 ± 0.946(06)	3.93 ± 0.672(15)
Female	4.53 ± 0.755(15)	3.00 ± 1.414(06)	4.53 ± 0.723(15)
Combined	8.80 ± 1.208(15)	5.83 ± 2.040(06)	8.47 ± 1.226(15)
PROPORTION OF PUPS BORN ALIVE	0.81 ± 0.099(15)	0.67 ± 0.211(06)	0.88 ± 0.080(15)
SEX OF PUPS BORN ALIVE (MALES/TOTAL)	0.46 ± 0.058(13) ^c	0.53 ± 0.105(04) ^c	0.44 ± 0.044(14) ^d
LIVE PUP WEIGHT (g)			
Male	1.69 ± 0.027(12) ^e	1.49 ± 0.050(04) ^f	1.75 ± 0.059(13) ^e
Female	1.66 ± 0.031(13)	1.62 ± 0.064(04)	1.67 ± 0.045(14)
Combined	1.68 ± 0.030(13)	1.54 ± 0.036(04) ^g	1.71 ± 0.049(14)
ADJUSTED LIVE PUP WEIGHT (g) ^h			
Male	1.72 ± 0.035(12)	1.45 ± 0.061(04) ^f	1.74 ± 0.033(13)
Female	1.69 ± 0.030(13)	1.58 ± 0.054(04)	1.66 ± 0.028(14)
Combined	1.71 ± 0.030(13)	1.50 ± 0.054(04) ^f	1.69 ± 0.029(14)

a: Mean ± SE.

b: Number of fertile pairs providing the data indicated in parenthesis.

c: Two litters in this group contained no live pups.

d: One litter in this group contained no live pups.

e: One litter in this group contained no live male pups.

f: Significantly different (p<0.05) from the control male X control female and 1.0% male X control female groups.

g: Significantly different (p<0.05) from the control male X control female group.

h: Least squares estimate of mean ± SE, adjusted for litter size (number of fertile pairs with live pups).

Table 8. Male Body and Organ Weights at Necropsy (Task 3)
Ethylene Glycol Monobutyl Ether

Variable ^a	Treatment Group	
	Control	0.5%
Body (g)	41.17 ± 0.9004(39) ^b	38.13 ± 0.7110(20) ^c
Liver (g)	2.049 ± 0.0512(39)	1.986 ± 0.0591(20)
R. Testis (g)	0.143 ± 0.0036(39)	0.132 ± 0.0032(20) ^c
R. Cauda (g)	0.021 ± 0.0005(39)	0.020 ± 0.0009(20)
R. Epididymis (g)	0.061 ± 0.0016(39)	0.059 ± 0.0018(20)
Prostate Gland (g)	0.043 ± 0.0018(39)	0.042 ± 0.0025(20)
Seminal Vesicles (g)	0.717 ± 0.0246(39)	0.651 ± 0.0214(20)
Kidneys (g) ^d	0.744 ± 0.0164(39)	0.806 ± 0.0332(20)

a: Mean ± SE.

b: Number of animals providing the data indicated in parenthesis.

c: Significantly different (p<0.05) from the control group.

d: The kidneys were weighed with the adrenal glands attached.

Table 11. Reproductive Performance of Second Generation Breeding Pairs
(Treated with Ethylene Glycol Monobutyl Ether)

Treatment Group	No. with Copulatory Plugs/ No. Cohabited	Mating Index (%) ^a	No. Fertile/ No. Cohabited	Fertility Index (%) ^b
Control Male X Control Female	17/20 ^c	85	16/20	80
0.5% Male X 0.5% Female	17/20 ^d	85	15/20	75

a: Mating Index (%) = $\frac{\text{No. with Copulatory Plugs}}{\text{No. Cohabited}} \times 100$

b: Fertility Index (%) = $\frac{\text{No. Fertile}}{\text{No. Cohabited}} \times 100$

c: Although not detected by direct means, 3 females were scored plug-positive based on delivery of litters.

d: Although not detected by direct means, 1 female was scored plug-positive based on the delivery of a litter.

Table 13. Second Generation Female Body and Organ Weights at Necropsy
(Task 4) Ethylene Glycol Monobutyl Ether

Variable ^a	Treatment Group	
	Control	0.5%
Body (g)	31.77 ± 0.6957(20) ^b	31.84 ± 0.4359(20)
Liver (g)	1.847 ± 0.0441(20)	1.973 ± 0.0541(20)
Kidneys (g) ^c	0.447 ± 0.0127(20)	0.545 ± 0.0138(20) ^d

a: Mean ± SE.

b: Number of animals providing the data indicated in parenthesis.

c: Kidneys were weighed with the adrenal glands attached.

d: Significantly different (p<0.01) from the control group.

CECATS/TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA: Submission # BEHQ-0992-12464 SEQ. A

TYPE: INT SUPP FLWP

SUBMITTER NAME: Union Carbide Corporation

INFORMATION REQUESTED: FLWP DATE: _____
 0501 NO INFO REQUESTED
 0502 INFO REQUESTED (TECH)
 0503 INFO REQUESTED (VOL ACTIONS)
 0504 INFO REQUESTED (REPORTING RATIONALE)
 DISPOSITION:
 REFER TO CHEMICAL SCREENING
 CAP NOTICE

VOLUNTARY ACTIONS:
 0401 NO ACTION REPORTED
 0402 STUDIES PLANNED/IN PROGRESS
 0403 NOTIFICATION OF WORKING WITH MSDS
 0404 LABEL/MSDS CHANGES
 0405 PROCESS/HANDLING CHANGES
 0406 APP USE DISCONTINUED
 0407 PRODUCTION DISCONTINUED
 0408 CONFIDENTIAL

SUB. DATE: 09/24/92 OTS DATE: 09/29/92 CSRAD DATE: 08/01/95

CHEMICAL NAME: _____

acetic acid, butoxy-

CASE: _____
111-76-2

unknown

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
0201 ONCO (HUMAN)	01 02 04	0216 EPICLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEM/PHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECO/AQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCUREL/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQUEST DELAY	01 02 04	0248 PRODUSE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PROD/COMP/CHEM ID	01 02 04	0251 MSDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	<u>OTHER</u>	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04	<u>Erythrocyte fragility (in vitro)</u>	
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0229 METAB/PHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0230 METAB/PHARMACO (HUMAN)	01 02 04		

TRIAGE DATA	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN	USE:	PRODUCTION:
CAS SR	<u>YES</u>	YES (DROP/PREFER)	<u>In Vitro</u>	LOW		
	<u>NO</u>	NO (CONTINUE)	<u>RAT</u>	MED	<u>In human 295 ppm</u>	
	<u>IN PLANNING</u>	REFR	<u>DOG</u>	<u>HIGH</u>	<u>(8hr2 exposure) without hemolytic effect</u>	
			<u>RBT</u>			
			<u>HUMAN</u>			

REMARKS: A variety of studies carried out from 1943 to 1983, involving mice, rats, rabbits, guinea pigs, dogs, monkeys and human subjects, showed no evidence for any alterations of the blood cell forming capacity of the bone marrow, other than hemolytic anemia. For humans safe exposure level appears to be 200 ppm. Current ACGIH level of 25 ppm. The NOAEL for inhalation exposure 25 ppm.