The sulfonium ion degraded in both the perfusate and the bile at pH values above physiological. The authors postulate that it hydrolyzed to tetrahydrothiophene. This compound then could be reabsorbed by the liver *in vivo* and oxidized to 3-hydroxysulfolane, a urine metabolite of busulfan observed by Roberts and Warwick (*Biochem. Pharmacol.* 1961, 6:217) in rats, rabbits and mice. (See Marchand *et al.* review 9 below for a schematic representation of these structures.)

The following chart shows the change in concentration of busulfan, total reactivity and the conjugate with time in the rat liver perfusate.

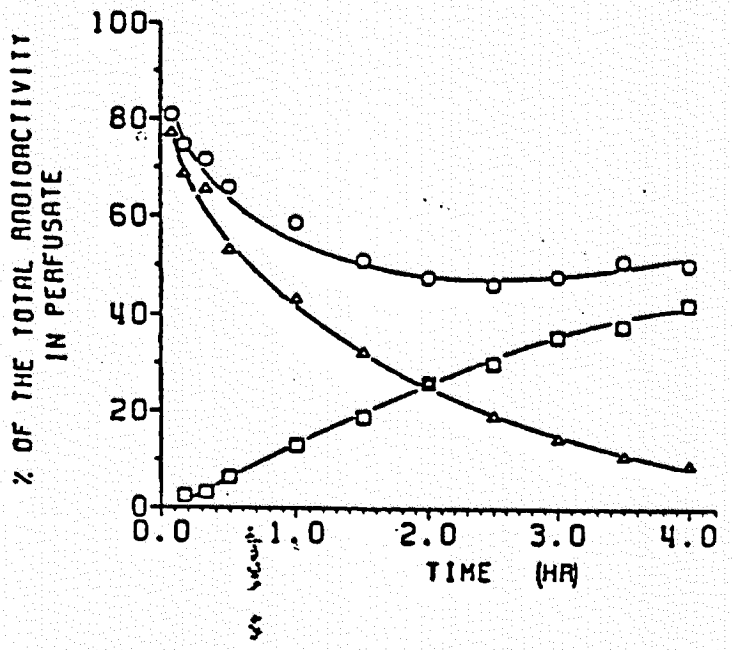


Fig. 5: Time course of ^{14}C-busulfan and its main metabolite in the perfusate as the percent of the total radioactivity administered.
O: Total radioactivity in perfusate.
Δ: ^{14}C-Busulfan in the perfusate.
□: The main metabolite of busulfan.

4) M. Hassan and H. Ehrsson, 1987a. Urinary metabolites of busulfan in the rat. Drug Metabolism and Distribution. 15(3):399-402. Volume 7, page 387.

This is a follow-up from the previous paper. The authors dosed male Sprague-Dawley rats with 15 mg/kg of ^{14}C-busulfan IP. They housed the animals in metabolism cages and collected samples for 72 hours (cumulative). They only recovered 69.2 ± 6.2% of the total radioactivity in the urine and only 1.5 to 2% in the feces. They did not measure the amount in the carcass, but again these results imply that a considerable amount of busulfan reacts nonspecifically *in vivo*.

HPLC analysis showed eight peaks in the urine samples. The authors identified the major peaks as unchanged busulfan (6% of total urine radioactivity), 3-hydroxysulfolane (39%), tetrahydrothiophene (20%) and sulfolane (13%) by GC/MS and NMR. These results support the author's hypothesis in the previous paper.

Lastly, the authors studied the toxicity of these major metabolites on Chinese hamster V79 cells *in vitro*. As expected only busulfan was cytotoxic at concentrations to 80 mM.

5) M. Hassan *et al.* 1988. Pharmacokinetics and metabolic studies of busulfan in rat plasma and brain. *European Journal of Drug Metabolism and Pharmacokinetics* **13**(4):301-305. Volume 7, page 392.

In the third paper in this informative series, the authors again dosed male Sprague-Dawley rats with 15-mg/kg ^{14}C-busulfan, IP. They took samples of plasma and brain at specified times to 8 hours and analyzed for busulfan and its metabolites by HPLC and GC. They found the same spectrum of metabolites in the plasma as in the urine, described above, and in approximately the same distribution. They also found a similar spectrum of metabolites in whole brain homogenate. The kinetic curve for busulfan in brain paralleled that of plasma with a near constant brain/plasma ratio of 0.74. The elimination half-lives in plasma and brain were 3 hours for intact busulfan and 8 hours for total radioactivity. Sampling was too short in these experiments, so I do not consider this last parameter accurate. The plasma protein binding was low, 9.2 ± 4.4%, and the octanol/water partition coefficient was 0.44 ± 0.08. The following chart shows the change in concentration of busulfan in plasma and brain with time.

M. Hassan et al., Busulfan pharmacokinetics & metabolism 303

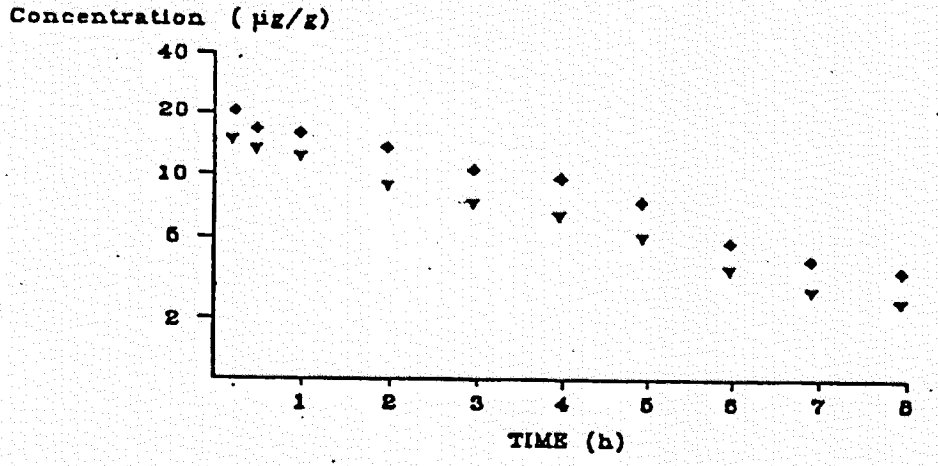


Fig. 1 : Plasma and brain concentrations following I.P. administration of busulfan (15 mg/kg) to the rat

conc. in plasma (◆)

conc. in brain (▼)

6) M Hassan *et al.* 1992. *In vivo* distribution of ^{11}-C-busulfan in cynomolgus monkey and in the brain of a human patient. *Cancer Chemotherapy and Pharmacology*, **30**:81-85. Volume 7, page 398.

In this fourth paper, these investigators gave ^{11}C-busulfan to cynomolgus monkeys and determined the distribution of the radioactivity by positron emission tomography. Over a one-hour examination the compound accumulated in the liver to concentrations 9-fold higher than those in the brain at the end of the experiment. Nevertheless, the experiment showed that busulfan rapidly crossed the blood-brain-barrier. The radioactivity peaked in both the cortex and the white matter within three minutes, but the amount in the cortex was 1.25 times higher than that in the white matter. This ratio declined to one after 30 minutes.

The authors also gave the compound to a single human patient, a 32-year-old woman with AML. In this patient, the busulfan concentration in the brain reached a maximum in five minutes with a cortex to white matter ratio of 1.6. Of the total dose, the investigators estimated that 20% entered the brain, consistent with total blood flow.

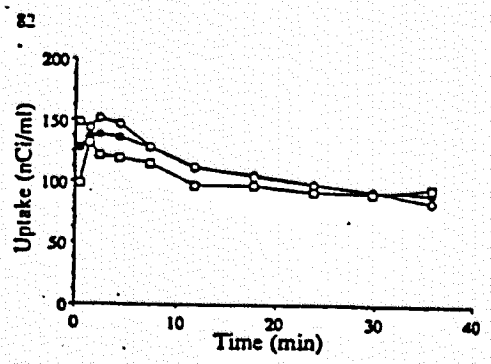


Fig. 1. Distribution of radioactivity in the monkey brain after an i.v. injection of 45 MBq [^{11}C]-busulfan as a function of time. ●, Frontal cortex; O, occipital cortex; □, white matter

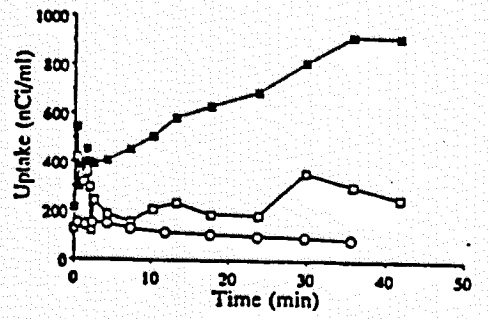


Fig. 2. Distribution of radioactivity in the brain of monkey 1 and in the liver and lungs of monkey 2 after the i.v. administration of 45 MBq [^{11}C]-busulfan to each monkey. O, Brain; ■, liver □, lungs

7) W. E. Fitzsimmons, *et al.*, 1990, Anti-convulsants and busulfan, *Annals of Internal Medicine*, **112**(7):552-553. Volume 7, page 314.

8) 8) W. E. Fitzsimmons, *et al.*, 1990. The effect of hepatic enzyme inducers on busulfan neurotoxicity and myelotoxicity, *Cancer Chemother Pharmacol*, **27**:226-228. Volume 7, page 317.

The first of these references is a short letter in which the authors respond to two previous letters describing the clinical use of anticonvulsants to prevent seizure secondary to high-dose busulfan therapy. The second reference describes in detail the results that engendered the warnings in the letter. The authors dosed female B6D2F1 mice with no pretreatment or pretreatment for three days before busulfan exposure with phenobarbital, phenytoin or Aroclor 1254. In the first set of experiments, they determined the effect of pretreatment on busulfan (100 mg/kg, single dose IP) neurotoxicity by assessing 24-hour mortality. Phenytoin and phenobarbital effectively prevented acute neurotoxicity in 60 and 68% of mice respectively. Aroclor did not. In a second set of experiments, they pretreated groups of mice as above, then treated the mice with busulfan (IP in 10% DMSO, 135 to 150 mg/kg body weight over six days, total dose). They then assessed 50-day mortality. An additional control group of mice received syngenic

bone marrow rescue after busulfan treatment. The following table shows the effect of pretreatment on 50-day survival.

Pretreatment	Dose	Number treated	Number Surviving	% Survival	Median days to death
None		37	8	22%	19
Bone Marrow Transplant		26	24	92%	21
phenobarbital	35 mg/kg/d	35	31	89%	24
phenytoin	15 mg/kg/d	20	0	0%	24
phenytoin	60 mg/kg/d	20	17	85%	8
Aroclor 1254	100 mg/kg PO	20	20	100%	NA

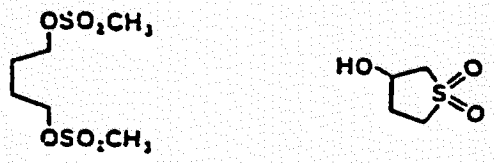
Both phenobarbital and phenytoin induce cytochrome P450 3A4 in humans. Cytochrome P450 3A4 is responsible for most sulfoxidation in humans. These compounds induce 2B1 and 2B2 in mice, enzymes also capable of sulfoxidation. Interestingly, Aroclor 1254, an inducer of CYP-1A1 and to a lesser degree 2B in mice, greatly increased 50 day survival, but had no effect on acute neurotoxicity. CYP-1A1 induction could not compensate for a high single dose of busulfan, but could greatly diminish the toxicity of smaller divided daily doses. These results suggest that pretreatment with anticonvulsants induces the metabolism of busulfan, thus lowering its neurotoxicity. It is possible that such pretreatment will decrease the effectiveness of busulfan therapy. Such pretreatment might also increase the potential for liver toxicity. Lastly, these results suggest that the large variability seen clinically in busulfan exposure (measured by AUC) may be a function of the widely variable induction states of human Cytochrome P450.

9) D. H. Marchand *et al.* 1987. Biliary excreation of a glutathione conjugate of busulfan and 1,4-diiodobutane in the rat. Volume 9, page 17.

Busulfan works as a potent alkylating drug because biological nucleophiles readily displace the methanesulfonate moieties by an SN_2 reaction (C. D. R. Dunn, 1974, *Exp. Hematol.* 2:101). Injected busulfan disappears rapidly from the plasma of mammals, only 10% of a radioactive dose remains in rat plasma after 10 minutes. (E. G. Trams *et al.* 1959, *Biochemical Pharmacology*, 2:7). In this early experiment, rats respired 12-14% of an IV radioactive dose as $^{14}CO_2$, excreted 1-3% in the feces and excreted 22 to 36% in the urine after 24 hours. Humans follow a similar excretion pattern after an IV dose (M. V. Nadkarni *et al.* 1959, *Cancer Res*, 19:713 and H. Vodopic *et al.*, 1969, *J. Lab. Clin. Med.* 73:266). In 0.05 M phosphate buffer, pH 7.0 at 37°, busulfan decomposes to form tetrahdrofuran with an apparent first order rate constant of 0.043 hr^{-1} (M. Hassan and H. Ehrsson, 1986, *J. Pharm. Biomed. Anal.* 4:95). This reaction accelerates with increasing pH. In one report, rats exhaled up to 20% of an IV dose as 1,4 butanediol (Trams, 1959).

Roberts and Warwick (1959, *Nature* 183:1509) showed that the rat excreted a single major urinary metabolite after an IV dose of busulfan. They later identified this metabolite as 3-hydroxytetrahydrothiophene-1,1-dioxide (3-hydroxysulfolane). But, they also observed that the sulfur from ^{35}S-busulfan was excreted quantitatively as methanesulfonic acid. This means that the sulfur of 3-hydroxysulfolane comes from an endogenous source. To determine this source these same authors

reacted busulfan with a number of sulfhydryl compounds (Roberts and Warwick, 1961, Biochemical Pharmacol. 6:205). When they reacted busulfan with cysteine at pH 8.0 three different conjugates formed, but only one in large quantity, the cysteine-sulfonium conjugate. When this conjugate is treated with base it hydrolyzes to form tetrahydrothiophene (THT, see the diagram below). The sulfur of this THT comes from the cysteine not busulfan. The result of this reaction is the liberation of two moles of methanesulfonic acid and the formation of THT from the butane backbone of busulfan. When these authors gave THT or the cysteine-sulfonium conjugate to rats, 3-hydroxysulfolane appeared in the urine, suggesting that these two compounds are intermediates in the metabolism of busulfan. These experiments also indicate that the oxygens of 3-hydroxysulfolane are not derived from the busulfan, but from subsequent oxidation of THT. This oxidation is probably hepatic and probably involves one or more cytochrome P450 reactions.



1 2

FIG. 1. *The structure of busulfan (1) and 3-hydroxysulfolane (2), the major urinary metabolite in the rat.*

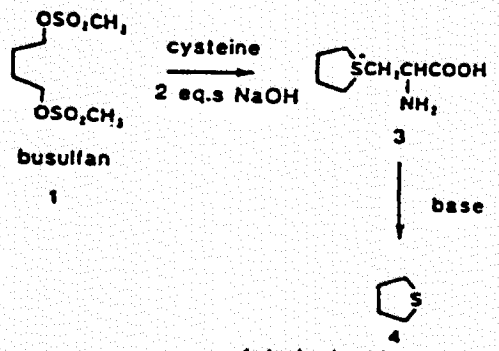


FIG. 2. *The reaction of L-cysteine with busulfan.*

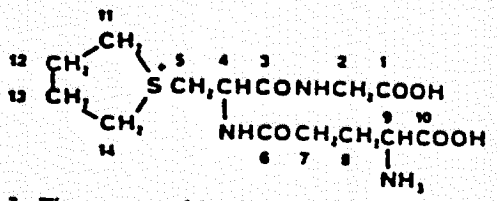


FIG. 3. *The structure of the glutathione sulfonium conjugate 5.*

Roberts and Warwick (1961) also observed that glutathione reacted *in vitro* with busulfan only when the pH of the reaction solution was greater than 8.0. Again, THT was the major product of this reaction with an active sulfhydryl.

In this research, Marchand *et al.* cannulated the bile duct of male rats under pentobarbital anesthesia. They injected the rats with 1.0 mg/kg busulfan and collected a series of 30-minute bile samples for 8 hours. They also synthesized the glutathione congjugate to serve as a standard. They then identified this conjugate in the bile of the cannulated rats by HPLC. They did not detect any cysteinylglycine, cysteine, or mercapturic acid metabolites in the bile. Previous research had shown that the glutathione-conjugate hydrolyzes to tetrahydrothiophene at high pH. Thus, the authors followed the time course of the excretion of the conjugate by measuring the concentration of THT by GC after the addition of base to the samples. After eight hours, 26 % of the dose was excreted in the bile as THT-releasing metabolites. The concentration in the bile peaked at 90 minutes. It decreased gradually over the eight-hour time course and was still detectable at the end of the experiment.

Pharmacology:

1) M. Cherian and U.M. Rawal. 1989. Effect of busulfan on crystalline lens- glutathione, glutathione reductase and glucose-6-phosphate dehydrogenase. *Indian J. Experim. Biol.* **27**(10): 915-916. Volume 7.

These authors dosed rats with 14 ppm busulfan in the diet (*ad libitum* feeding). At three and six months they killed groups of treated rats and controls and analyzed the lens for cataracts, glutathione concentration, glutathione reductase activity, and glucose 6-phosphate dehydrogenase (G-6-PD) activity. Glutathione reductase reduces oxidized glutathione to reduced glutathione. G-6-PD forms NADPH in the hexose monophosphate shunt. NADPH is a substrate for glutathione reductase. Cataracts did not form by three months, but formed consistently in treated rats by six months. The following table shows the results of the tissue assays.

Parameter	units	Control	sd	3 months treatment	sd	control	sd	6 months treatment	sd
GSH	µmol/g tissue	4.84	0.02	5.22	0.34	4.34	0.22	1.07	0.07
Glutathion reductase	units/g tissue/hr	6.41	0.13	11.47	0.54	5.64	0.37	1.66	0.2
G-6-PD	units/g tissue/hr	38	0.48	33.6	1.2	41.7	0.6	10.1	0.6

The results suggest that the cataracts caused by low dose busulfan are not the result of direct depletion of glutathione, but of the suppression of the enzymes involved its cyclic reduction. The authors do not postulate whether this suppression is the result of direct damage to the enzyme, decreased expression or simple cumulative damage to the cells. A decrease of G-6-PD by one third is a profound cellular derangement.

2) P Grimes and L. Von Sallmann, 1966. Interference with cell proliferation and induction of polyploidy in rat lens epithelium during prolonged myleran treatment. *Experimental Cell Research*, *42*(2):265-273. Volume 7, page 342.

Grimes and Von Sallmann fed groups of young male rats a diet containing busulfan for 2, 3, 4, and 7 days, 2, 4, 6 and 8 weeks. They killed the groups at these different times and examined the rat's eyes. The dose range for all rats was 1 to 2.5-mg/kg/day. Total number of cells in mitosis declined with time. They observed that prolonged administration of busulfan interfered with cell proliferation in the lens epithelium by inhibiting mitotic division in cells that have completed DNA synthesis. Repetition of this process in successive cell cycles led to severe depletion of the epithelial population and to the appearance of cells of increasingly higher ploidy.

3) A. E. Light. 1967. Additional observations on the effects of busulfan on cataract formation, duration of anesthesia, and reproduction in rats. Toxicology and Applied Pharmacology **10**(3):459-466. Volume 8, page 183.

"Recently weaned rats developed cataracts within 10 weeks after being given a diet containing 15 ppm of busulfan ..... . However, when the drug was introduced at 90 days of age for 21 weeks, cataracts did not develop until after some additional 9-18 weeks on a drug-free diet. Rats exhibiting toxic symptoms produced by busulfan had prolonged hexobarbital sleeping-times and for a few, the combination was even lethal. Busulfan likewise interfered with reproduction at this dosage by reducing the number of pups in litters, increasing fatalities in female parents, and probably interfering with sperm formation in the males."

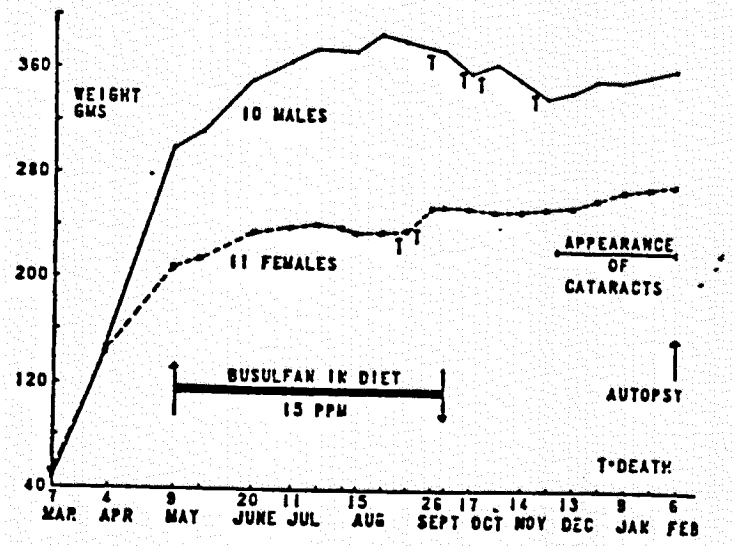


FIG. 1. Delayed appearance of cataracts in rats fed busulfan at a concentration of 15 ppm in the diet, starting at 12 weeks of age.

4) R. B. Epstein *et al.* 1992. A canine model for hepatic veno-occlusive disease. *Transplantation.*, **54**:12-16. Volume 7, page 281.

These authors were searching for a consistent model for veno-occlusive disease (VOD) in the dog. They tried monocrotaline, busulfan and radiation. Monocrotaline produced VOD most severely and consistently. Nevertheless, six dogs treated with 2 mg/kg/d oral busulfan for 17 to 25 days all developed microscopic liver damage. This included central congestion, zone 3 necrosis, focal venous destruction, hemorrhage, and venous fibrosis. Four of the dogs died or were moribund between day 17 and 25. Importantly, the dogs did not show changes in Liver Function Tests.

5) H. P. Lohrmann and W. Schreml, 1982. Cytotoxic drugs and the granulopoietic system. *Recent Results in Cancer Research* **81**:1-22. Volume 8, page 210.

This is a weighty and interesting review of both clinical and non-clinical information. I will not attempt to comment on all its many details. Nevertheless, in a section describing bone marrow changes, the author cites the work of Dunn (*J Natl Cancer Inst.* 1974, 52:173-180) saying that after a single dose of busulfan, femoral cell content decreased to 40% by 24 hr and 9% by 48 hr (I assume in either rats or mice). Regeneration of femoral cells had begun by 96 hours after dosing.

In this same section the author cites H. Simpson (*Br. J. Heamatol* 1977, 35:459-464) saying that these investigators determined an inverse linear relationship between busulfan dose and marrow cellularity. These authors also "observed an impressive circadian variation of the effect of Melphalan (sic) on the reduction of marrow cellularity." They observed that busulfan was most effective when given during the dark period.

Mutagenicity and Genotoxicity:

1) International Agency for Research on Cancer (IARC), 1,4-butanediol dimethanesulphonate (Myleran). IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: Chemicals, Industrial Processes and Industries Associated with Cancer in Humans, 1982; Suppl 4:68-71. Volume 8, page 27.

This monograph is succinct, and is a good description of the carcinogenic risk associated with busulfan so I will quote part-A almost verbatim.

A. Evidence for the carcinogenicity to humans (sufficient)

"Many cytological abnormalities, including giant nuclei, cytomegaly and dysplasia have been observed in leukemia patients treated with Myleran; in one instance, cytomegaly was seen in an infant who was *in utero* during treatment of the mother. A patient treated with Myleran for polycythemia vera

developed acute and chronic heamolysis, pancytopenia, erythroid hyperplasia, dyserythropoiesis, and circulating normoblasts, all of which reverted to normal after cessation of Myleran therapy. Carcinomas of the breast and of the vulva have been seen in association with similar cytological abnormalities in the respective solid tissues. One case of acute non-lymphocytic leukemia has also been reported in a patient with polycythemia vera treated with myleran. After surgical removal of all visible intrathoracic lung cancer, 726 patients in London were allocated at random to treatment with Myleran (243), cyclophosphamide (234) or placebo (249). After five years, 69 patients who received myleran, 63 who received cyclophosphamide and 85 who received placebo were still alive. By nine years, pancytopenia had developed in 20 of the patients, 19 of whom had received Myleran; of these 19; four developed and died from acute nonlymphocytic leukemia. While none of these were among the 15% of patients treated with myleran who also received radiotherapy or other cytotoxic drugs or both, no relationship was observed between cumulative dose of myleran and either pancytopenia or leukemia. Solid tumors occurred in 19 other patients in the study but were approximately equally divided among the three drug groups."

I will not give the original source data here. For those references please see the monograph. The monograph also contains short summaries of animal data and short-term test that demonstrate the carcinogenic potential of myleran. I will not quote these here as the human data is sufficient to establish myleran as a carcinogen. Nevertheless, this preclinical information shows that myleran is mutagenic, clastogenic and carcinogenic in appropriate model systems.

Busulfan "was positive in a test for DNA damage and was mutagenic in *Salmonella typhimurium*, *Drosophila melanogaster* and barley. Myleran induced chromosomal aberrations in barley and in intact rodents" (Chinese hamster and mouse) "and chromosomal aberrations and sister chromatid exchange in cultured human cells" (normal lymphocytes). "It induced dominant lethal mutations in a teleost fish and in rodents" (mouse oocytes) "but was negative in the mouse specific locus test. It induced cell transformations in BHK cells. Myleran induced chromosomal aberrations and sister chromatid exchanges in lymphocytes and bone marrow cells from patients treated with this drug for chronic myeloid leukeamia."

The following table summarizes these results.

	DNA Damage	Mutation	Chromosomal Anomalies	Other
Prokaryotes	+	+		
Fungi/Green plants		+	+	
Insects		+		
Mammalian cells (*in vitro*)	+	+	+	Cell transformation
Mammalian cells (*in vivo*)		+	+	Dominant Lethal mutations
Humans (*in vivo*)			+	Secondary Leukemia

Reproductive Toxicity:

1) G. F. Jansz and D. K. Pomerantz, 1984, The effects of prenatal treatment with busulfan on *in vitro* androgen production by testes from rats of various ages. *Can J Physiol Pharmacol* 1985; 63(9):1155-1158. Volume 8, page 46.

These researchers treated pregnant Sprague-Dawley rats with 10 mg/kg of busulfan or vehicle control (peanut oil) on day thirteen of gestation. They retained and randomized the male pups. At 12, 30, 60 and 90 days after parturition they killed groups of the pups and measured body weight, testis weight, serum androgen (by RIA) and serum lutenizing hormone (LH, by RIA). They also harvested testis tissue and used this tissue to measure *in vitro* androgen production with and without LH stimulation. They reported this information as ng-androgen per gram of tissue, ng-androgen per testis (the androgen per gram of tissue multiplied by the testis weight) and ng androgen per million Leydig cells.

Male F1 rats from busulfan treated dams weighed less than controls at all time points (35% less than control on day 12, 18% less than control on day 90). Testis weight was also greatly reduced in treated rats (43% of control on day 12, 21% of control on day 90). So the testis continued to atrophy as the rats matured. Serum androgen concentration was 26% less than control on day 90. Serum LH concentration was two fold greater than control on day 90. The latter response is homeostatic compensation by the anterior pituitary.

At 12 days androgen concentration in testis tissue was severely decreased (14% of control). LH stimulation returned these concentration to almost normal. By 90 days the testis were recovering the ability to synthesize androgen (83% of control) but the effect of LH was less pronounced. Paradoxically, androgen concentrations in Leydig cells were higher in cultures from busulfan treated rats (>2 fold on day 60, 20% higher on day 90). Concentrations after LH stimulation were as much as 3-fold higher in cultures from treated animals (day 60). The authors suggest that there may be fewer Leydig cells in the testis of treated rats and these cells are compensating, so when they are isolated the concentration is higher. But, this unusual result also could occur if busulfan is cross-linking the LH receptors at the cell surface and thereby increasing the signal for androgen production.

TABLE 3. *In vitro* androgen production by testicular fragments, testis, and Leydig cells (LC) from rats treated prenatally with busulfan

Rats	Androgen (ng/mg tissue)		Androgen (ng/testis)		Androgen (ng/10^6 LC)	
	Basal	Stimulated	Basal	Stimulated	Basal	Stimulated
12 days old						
Control	0.141±0.033[a] (5)	1.246±0.133 (5)	2.27±0.45 (5)	24±3 (5)	—[c]	—
Busulfan	0.037±0.003[b] (6)	2.990±0.240[b] (7)	0.33±0.03[b] (6)	23±2 (7)	—	—
30 days old						
Control	0.116±0.017 (10)	1.340±0.074 (10)	42±6 (10)	494±38 (10)	—	—
Busulfan	0.210±0.022[b] (10)	2.536±0.138[b] (10)	27±3[b] (10)	309±17[b] (10)	—	—
60 days old						
Control	0.313±0.032 (21)	1.419±0.135 (21)	411±44 (21)	1853±178 (21)	4.67±0.15[d] (12)	42.63±2.04 (12)
Busulfan	0.670±0.046[b] (22)	2.756±0.452[b] (21)	209±11[b] (22)	801±111[b] (21)	11.26±0.31[b] (12)	126.15±2.29[b] (12)
90 days old						
Control	0.147±0.016 (11)	1.122±0.112 (11)	213±25 (11)	1625±163 (11)	8.56±0.18 (15)	75.42±2.94 (15)
Busulfan	0.540±0.071[b] (11)	3.954±0.502[b] (11)	177±25 (11)	1304±249 (11)	10.52±0.33[b] (15)	156.15±5.82[b] (15)

[a] Values expressed as mean ± SEM with the number of observations in parentheses.
[b] Significantly different ($p < 0.05$) from the control.
[c] Not measured.
[d] For all dispersed LC preparations, the number of replications of cell incubations from at least 10 animals is in parentheses.

From a regulatory point of view, busulfan is particularly toxic to male mammals *in utero*. Giving busulfan to pregnant women could severely compromise the growth, development and reproductive capacity of a surviving male child.

2) Z. Krawczyk and N. Szymik 1989, Effect of age and busulfan treatment on the hsp70 gene-related transcript level in rat testes. *International Journal of Andrology* **12**:72:79. Volume 8, page 155.

The authors used Northern Blot to determine the expression of a testes specific heat shock gene (hsp70)-related transcript (hst70 RNA) in testicular tissues of rats. Their method was only semi-quantitative. They found that expression of this transcript decreased with age to approximately one year then the expression stabilized in rats that retained testicular function. Testicular tissue from rats with completely atrophic testes did not express this protein.

Two-month-old rats treated with a single IP dose (10 mg/kg) of busulfan lost testicular function and did not express the transcript. Four weeks after the injection "the seminiferous tubules still contained spermatids but very few spermatocytes. Six weeks after administration of busulfan the seminiferous tubules were depleted of spermatocytes and spermatotids. The expression of the transcript declined to undetectable levels. Recovery was only partial eleven weeks after the injection. Busulfan inhibits spermatogonial mitoses while the function of other testicular cells is relatively unaffected.

PEG:

1) I. Berenblum and N. Haran, 1955, The influence of croton oil and of polyethylene glycol-400 on carcinogenesis in the forstomach of the mouse. *Cancer Res.*, **15**(7):510-516. Volume 7, page 132.

In this ancient paper, these authors were attempting to demonstrate the two step model of carcinogenesis in a mouse forestomach (squamous epithelium) model as it had been previously demonstrated in a mouse skin model. Their results were indeed interesting and unexpected and the paper is worth the read, but I will confine my review to the results they observed with PEG-400, the solvent for all test chemicals (initiators) and the promoter, croton oil. In the relevant PEG experiments, fasted mice were dosed, PO, with a single dose of an initiator, then dosed once per week with PEG, again PO. In three of four cases, PEG-400 seemed to prevent the formation of tumors, but only in the promotion phase. It did not inhibit the formation of tumors associated with the dosing of the initiator, but continued dosing throughout the promotion period, 30 weeks, greatly decreased the tumor yield. The following table shows these results. In this table BP = 3,4-benzpyrene, DMBA = 9,10-dimethyl-1,2-benzanthracene, MCA = 20-methylcholanthrene, and DBA = 1,2,5,6-dibenzanthracene.

Primary treatment carcinogen	Secondary treatment	Tumor Yield	p	Percent Tumor Yield
	No TX – PEG +	incidence/N		
BP	–	17/20		85
	+	11/24	<0.001	46
DMBA	–	29/41		71
	+	5/20	<0.001	25
MCA	–	20/25		80
	+	2/13	<0.001	15
DBA	–	0/20		0
	+	2/20	>0.2	10
PEG alone		0/20		0

These results are difficult to interpret, but it is possible that PEG is suppressing dedifferentiation.

2) W. Bartsch *et al.* 1976. Acute toxicity of various solvents in the mouse and rat. *Arzneim.-Forsch (Drug Res.)* **26**(8):1581-1583. Volume 7, page 129.

These investigators did standard LD_{50} determinations using probit analysis of a number of standard solvents. The following table shows the results for PEG-400.

PEG-400	Mouse			Rat		
	IV	IP	PO	IV	IP	PO
LD_{50} ml/kg	7.6	12.9	26	6.5	13.1	30
95% confidence	7.3-7.9	11.6-14.2	21.6-31.2	6.3-7.1	12.1-14.4	
Slope	1.07	1.12	1.45	1.09	1.17	
specific gravity	1.125					
LD_{50} g/kg	8.6	14.5	29.3	7.3	14.7	33.8
LD_{16} g/kg	8.0	13.0	20.2	6.7	12.6	

3) R.L. Carter, 1969. Early development of injection-site sarcomas in rats: a study of tumors induced by a rubber additive. *The British Journal of Cancer*, **23**(2): 408-416. Volume 7, Page 213.

Dr. Carter studied the carcinogenic potential of a rubber additive, polymerized N-nitroso-2,2,4-trimethyl-1,2-dihydroquinoline or NTDQ, given by three routes, SC, IP and PO. As in other studies I have reviewed here, he used PEG-400 as the vehicle and as a vehicle control. He dosed groups of 40 rats with 25 mg NTDQ in 0.25 mg of PEG-400 or with 0.25 ml of PEG-400 (SC only) once per week for ten weeks on the right flank than once per week for 10 on the left. He followed the animals for 100 weeks. PEG-400 dosing caused no tumor and no microscopic abnormalities. Nevertheless, the dosing here is probably inadequate to be a true test of PEG-400 carcinogenicity.

4) F. J. C. Roe *et al.* 1966. Carcinogenicity of certain glycidyl derivatives. *Food and Cosmetics Toxicology* **4**(3):365-368. Volume 124, page 124.

Again, this is a study of various chemicals in male mice where the test compounds are dissolved in PEG-400. The compounds are mono-functional epoxides and PEG-400 alone was tested as a control. Mice received weekly injections of PEG-400 (0.2 ml) in the right flank for 52 weeks with a four-month follow-up. These mice developed no injection site tumors. The authors describe no toxicity in this control group. The epoxides tested did cause slight increases in the number of tumors at the injection site.

5) H. F. Smyth *et al.* 1947. The toxicity of high molecular weight polyethylene glycol; chronic oral and parenteral administration. *J Am Pharmaceut. Assoc.* **36**:157-160. Volume 9, page 155. Volume 9, page 155.

6) H. F. Smyth et al. 1955. The chronic oral toxicology of the polyethylene glycols. *J. Am. Pharmaceut. Assoc.* **44**(1):27-30. Volume 9, page 160.

7) H. F. Smyth *et al.* 1950. The toxicology of the polyethylene glycols. *J. Am. Pharmaceut. Assoc.* **89**:347-354. Volume 9, page 165.

I shall review these three papers as a whole. The last paper is a review plus new data. Smyth *et al.* determined the toxicity of various polyethylene glycols in rats, rabbits and guinea pigs. They also compared these values to those that Dr. Smyth's group had reported in 1941 and 1945 (Smyth *et al.* 1941, *J. Ind. Hyg. Toxicol* 23:259 and Smyth *et al.* 1945, *The Journal* 34:72). Evidently, their purpose was to monitor changes in toxicity among production runs of PEG in successive years. There was some variability. The values from the earlier work tended to be lower than those determined in 1949. Dr. Smyth postulates the existence of an impurity in earlier batches of various PEG compounds. Thus, the higher values should be the most relevant today.

The oral (gavage) LD_{50} for PEG-400 in the male rat ranged between 30.2 and 43.6 g/kg. These values are in the same range as that later reported by Bartsch *et al.* (above). In the Guinea pig, the oral LD_{50} for PEG-400 was 15.7 g/kg and in the rabbit it was 26.8 g/kg. Dr. Smyth's claims that the toxicity of the polyethylene glycols decreases slightly with increasing molecular weight, but the differences are small above PEG-200. Rats that succumbed to a single dose of PEG "die with renal lesions similar to those resulting from smaller amounts of ethylene and diethylene glycols. Cloudy swelling of the liver parenchyma is also found."

In a 1947 experiment, this group provided PEG-200, 300 and 400 to rats as a 16% solution in the drinking water for 90 days (11 to 20 g/kg/day). This dose cause a decrease in water consumption and two thirds of the animals died before the end of the experiment. These animals suffered "necrosis of the renal tubular epithelium", but surviving animals showed little damage. A dose of 4% in the drinking water (approximately 5 g/kg) caused no ill effects. In a 1948 experiment, 16% PEG-400 killed all the rats within 13 days. Doses of 8% and 4% caused only a slight decrease in kidney weight.

In a 1947 experiment, a daily IV dose of one gram of PEG-400 as a 5% solution in saline (six doses per week for five weeks) killed one of five rabbits. Assuming a 3-kg rabbit this is approximately 333 mg/kg/d for thirty days. The animal that died had cloudy swelling in the liver and kidneys. The authors subsequently found that the injection solutions were contaminated with 0.02 % or less formaldehyde. This admittedly small amount of this potent toxin was probably enough to confound this otherwise adequate experiment.

The investigators gave doses of 0, 1, 2, 4 and 8% PEG-400 fed to rats in their diet for two years. The high dose caused the male rats to gain weight more slowly than controls. Four mongrel dogs fed 2% PEG in their diet for a year suffered no toxic symptoms and showed no hematological, gross or microscopic differences from controls.

In skin penetration tests, the authors showed that one need practically dip a rabbit in PEG and leave it there for 24 hours in order to kill it (20 ml/kg PEG-400 killed 0 of six using the cuff test). Repeated inunction with PEG-400 caused no local irritation. A 20% solution caused no corneal injury. Eight repeated intracutaneous injections of PEG 400 caused some immune sensitization in 62% of tested guinea pigs (mild edema). Nevertheless, PEG-400 caused no sensitization in a 1949 human skin patch test (100 male and 100 female volunteers).