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DIMETHYL ETHER: A SAFETY EVALUATION

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Dimethyl ether (DME) has been used as an aerosol propellant for over 50 years. Its importance has markedly increased in the United States and Western Europe within the last decade and has prompted extensive toxicologic evaluations. In a number of animal studies, DME has shown a low order of acute and chronic toxicity, and no mutagenic, teratogenic, or carcinogenic behavior.

Based on results from a recently completed animal lifetime inhalation study, the Du Pont Company has approved the use of Dymel A propellant (dimethyl ether) for general aerosol usage, including personal products.

In this paper, we are presenting a safety evaluation for dimethyl ether propellant, not only from the viewpoint of the results of toxicity tests carried out, but also from the viewpoint of the objectives of a typical Du Pont toxicology program. These objectives include the implementation of the specific tests we feel are important in various stages of the development and commercialization of a product and how these toxicity tests relate to commercial status. We will, also, in this discussion, present new information on the toxicity of dimethyl ether propellant and the business implications of this new information.

Toxicity Program Objectives

The principal objective of toxicity work in Du Pont's Freon Products Division is to provide information on our commercial products for continued substantiation of their safety-in-use. A second objective is to determine if candidate development compounds can be safely used in our general business areas, which include propellants, solvents, blowing agents, and refrigerants. Specifically, we in the Freon Products Division and personnel from Haskell Laboratory work jointly to establish a priority list for testing which is based on business potential and the extent of human exposure. Protocols are developed, the necessary authorizations are obtained, timetables are set, the work is done, and results are reported in the usual fashion. Importantly, there are business implications which are based on toxicologic findings and these are provided to management.

Typical Toxicity Testing Program

Table I lists a typical toxicity testing program for an aerosol propellant. This program may not be the same for Freon Division prod-

TABLE I
Typical Toxicity Testing Program for an Aerosol Propellant

Level 1—Completed early in product and process evaluation of a compound
- Acute inhalation—establishment of ALC or LC_{50}
- Mutagen—bacterial study (Ames)—indicator of DNA interaction potential
- Skin and eye—determination of irritation/sensitization

Level 2—completed prior to serious market development
- Subchronic inhalation—preliminary measure of systemic toxicity (two-week test)
- Cardiac sensitization—determination of tendency to sensitize heart to epinephrine (Adrenalin)

Level 3—completed prior to commercialization
- Inhalation teratology—determination of developmental toxicity (embryotoxic/teratogenic effects)
- Prolonged inhalation—more complete measure of systemic toxicity (90-day test)

Level 4—completed or in progress prior to commercialization for certain uses
- Lifetime inhalation—determination of carcinogenicity, chronic toxicity, effect on life span (two-year test)

ucts that are directed to other uses or for other compounds that are developed, tested, and commercialized by other divisions of the Du Pont Company. We developed this protocol about a decade ago in our search for alternative compounds during the initial phases of regulation of chlorofluorocarbons by federal agencies. It's a dynamic program, not a static program. As new tests are developed within and outside Du Pont, we continue to add evaluations that we believe are essential for the safety-in-use of a compound as it proceeds from the research and development stage to commercialization or, for that matter, for compounds that are commercial and require additional testing. Not surprisingly, this toxicity program is heavily oriented toward inhalation studies because that is the principal route by which humans will be exposed to an aerosol propellant.

For a new compound, for example, we require the development of an ALC (approximate lethal concentration) or LC_{50}, some *in vitro* mutagen testing, and skin and eye irritation and sensitization to be completed early in the product and process evaluation of the compound. This information is important, first, because it relates to the exposure of our own employees to the compound at an R&D level, and, second, it is an indication of potential commercial value from a toxicologic viewpoint. Obviously, if a commercial compound exists that we would like to market as an aerosol propellant, these tests will be critical at an early stage in our interest.

Level 2 testing, which should be completed prior to serious market development, involves a subchronic inhalation study and the determination of the cardiac sensitization threshold for the compound. Since the cardiac sensitization phenomenon was first recognized 15 to 20 years ago, this testing finds its way into nearly every Freon Division Protocol for product testing.

Prior to commercialization we need information on developmental toxicity as it relates to possible embryotoxic or teratogenic effects. We also need advanced systemic toxicity testing and that, generally, will be in the form of a 90-day inhalation study.

And, finally, for an aerosol propellant, a lifetime inhalation in animals is necessary for the determination of the absence of carcinogenicity, a complete picture of chronic toxicity, and the effect on mammalian life span.

Physical Properties

Table II shows some of the essential properties of DME. Dimethyl ether is a high-pressure propellant with a vapor pressure of about 63 psig at 70°F. It is the most water-soluble, liquified gas propellant available in the world today with a solubility in water of 34 wt. % at 70°F under autogenous pressure. Its density is low, as would be expected from its chemical structure. It is a very strong solvent with a kauri-butanol value of 60, and is flammable with explosion limits ranging from 3.4 to 18%.

Prior to 1982 Dymel A was not commercially available in the United States. DME was commercially available from a number of companies in Europe and Japan. Many of the toxicity tests outlined in Levels 1, 2, and 3 were completed or in progress.

In December 1982 Du Pont made dimethyl ether available as Dymel A propellant for aerosol insecticides, paints and coatings, household, industrial, and automotive uses in the United States. From our viewpoint, this was possible because Levels 1, 2, and 3 testing had been completed and satisfactory results had been obtained. Our internal philosophy was not to commercialize Dymel A for personal products such as hair sprays or antiperspirants until we had completed a lifetime inhalation study in animals. We began a chronic inhalation study in rats (4) in May 1982 for the purpose of obtaining data in Level 4. Because that study has been satisfactorily completed, Dymel A propellant is now commercially available as a general aerosol propellant.

Testing for Toxicity

Before explaining the toxicology program developed for di-

TABLE II
Dimethyl Ether—Physical Properties

Formula	CH_3OCH_3
Molecular weight	46.07
Boiling point (F)	−12.7
Vapor pressure (psig)	
70°F	63
130°F	174
Solubility in water 70°F—autogenous pressure (wt%)	34
Density (g/cc) 70°F	0.66
Kauri-butanol value	60
Flammability limits in air (vol. %)	3.4–18

methyl ether, we want to redefine the word "risk assessment." At Du Pont, we prefer to use the word "hazard assessment," because risk assessment becomes a buzzword that too frequently is made synonymous with the evaluation only of cancer potential. In our laboratories, through the program from Levels 1 to 4, we were concerned with not only cancer but all of the other biological events that can occur that we would prefer not to occur. Our job was 1) to define levels at which events occurred and 2) to determine what tissues or organs first responded for reasons of setting limits, both internal and external, for handling the material safely.

Level 1. At Level 1 we really were getting into the basics for this material, finding out how much material it takes to produce a response using standard tests such as LC_{50} or approximate lethal concentration studies. Table III shows that in 15-minute exposures to mice or four-hour exposures to rats, you need atmospheres of 16% to 49% of dimethyl ether before any mortality occurs. The dose response is rather steep and the approximate lethal concentration really approaches these numbers.

The major sign of response to dimethyl ether, as one might expect, is sedation and narcosis as the levels get higher up to a point where mortality occurs.

TABLE III
Level 1 Testing

Acute inhalation toxicity
- LC_{50} Mice 1/4 hr 490,000 ppm (49%)
  1/2 hr 380,000 ppm (38%)
  Rats 4 hr 164,000 ppm (16.4%)
- Signs of response: sedation; narcosis

Skin and eye irritation/sensitization
- DME exists as a gas; skin/eye contact only from vapor phase

Mutagenic potential
- Not genetically active in salmonella assay either with or without metabolic activation
- Not active in V79 Chinese hamster cells (gene mutation)
- No increase in DNA-repair synthesis-rat liver cells
- Not active in sex-linked recessive lethal mutation assay (*Drosophila*)
- Not active in host-mediated assay in mice

TABLE IV
Level 2 Testing

Cardiac sensitization
- Weak cardiac sensitizer in dogs
  5-min exposure to 200,000 ppm produced response in two of 12 dogs
  5-min exposure to 300,000 ppm produced response in two of six dogs
  Signs of DME toxicity seen at this level

Subchronic inhalation toxicity
- Rats, 6 hr/day, 5 days/wk × 2 wk (+ recovery)
- 0, 10,000, 50,000 ppm
- 10,000 ppm—slight sedation
- 50,000 ppm—sedation, body weight gain suppression, hematologic and organ weight changes, no histopathologic organ changes
- All changes reversible
- Rats, 6 hr/day, 5 days/wk × 4 wk
- 0, 100, 1,000, 10,000 ppm
- No toxicologic changes
- Hamsters, 6 hr/day, 5 days/wk × 4 wk
- 0, 2,000, 10,000, 20,000 ppm
- No toxicologic changes

Because the material exists as a gas, studies of skin and eye-irritation were not conducted, although in animal exposure systems one can measure to some extent the amount of eye/skin damage to an animal that's been exposed to a gas.

Early in our Level 1 testing, we looked at the possibility of this material having serious biological activities, perhaps the ability to interact with macromolecules such as deoxyribonucleic acid (DNA). We did a series of mutagenic studies to measure the potential of DME to produce genetic damage (Table III). We studied at least five bioassay systems all the way from bacterial systems such as those used in the Ames assay to in vivo systems where we used mice exposed to high, but survivable, concentrations of DME; (in these studies we used indicator organisms that gave us enhanced sensitivity to determine whether the material could or could not interact with DNA to produce genetic damage). We found DME to be inactive in all of these test systems.

Level 2. We looked at the ability of this material to produce cardiac sensitization. This phenomenon is seen with many halogenated hydrocarbon materials. We found that DME was, at worst, a weak sensitizer in dogs (Table IV). Doses of 20 or 30% for five-minute exposures were able to sensitize a small percentage of the exposed dogs. We got a dose response and at those levels the dogs were definitely sedated, maybe to the point of narcosis. However, the material is a weak cardiac sensitizer compared to materials such as chloroform, carbon tetrachloride, and benzene, which are more potent cardiac sensitizers.

In the classical toxicology program, we looked at the biological response of rats to short-term exposures (Table IV) at concentrations up to and including 5% or 50,000 parts per million (ppm). As you exceed 50,000 ppm for extended periods of time, the amount of sedation seen becomes extensive and the animals are asleep most of the time. At 10,000 ppm the only sign of a response was slight evidence of sedation, the spontaneous movement in these animals being somewhat less than seen in unexposed animals.

Also at 50,000 ppm, the animals grew less well than did their untreated counterparts, and we saw the beginnings of hematologic and organ weight changes. The hematology changes were minor: a small increase in the number of leukocytes, perhaps a decrease in

the number of red blood cells. Kidney and liver weights appeared to be somewhat increased on a body weight basis. This finding may indeed reflect more the fact that the animals grew less quickly than did the controls. That conclusion is strengthened by the fact that after these exposures, looking at the tissues under a microscope, there was no evidence of any histologic damage in any of the tissues. All of the changes seen during the two-week exposure period were completely reversed following a two-week recovery period.

We followed this experiment by extending the exposure period. The animals were treated with doses of zero, 100, 1,000, and 10,000 ppm for four weeks. We saw no evidence of a change in these rats. We conducted the same experiment in hamsters with exposure levels of 2,000, 10,000, and 20,000 ppm (Table IV). In that experiment there were no toxicologic changes, particularly no signs of sedation.

Level 3: Pharmacokinetics. We then looked at the pharmacokinetics of the material (Table V). Using radiolabeled compound, rats inhaled the material for up to six hours. Steady state in tissues and organs is reached within a half hour. When the rats are removed from DME exposure, the tissue declines, an exact mirror of the tissue uptake. They rapidly go back to background levels with biological half lives in blood described by a two-phase system—ten minutes for the α phase, 90 minutes for the β phase. The most important result in this experiment was that no tissue storage was seen, which is what you would expect from a material that rapidly enters and rapidly leaves the body.

Concentrations tested range from 750 to 2,000 ppm and all of the statements above hold for that range of concentrations.

Level 3: Developmental Toxicity. At Level 3, we looked at the potential of this material to be a developmental toxin, that is, to interfere with the successful reproduction cycle of a female animal. We used our animal model, the rat (Table V), exposed during Days 6 through 15, which is the period of rapid organogenesis in this species: six-hour-a-day concentrations ranging from 1,250 to 40,000 ppm.

In these tests, one looks at the maternal animal in terms of overall response, not really toxicologic response other than body weight gain. In other words, one doesn't do a complete histopathologic examination on these animals, but focuses particularly on the fetuses, looking for congenital malformations and/or embryo mortality produced as a result of exposure looking very carefully at the skeletal structure, internal development of the organs, and gross outer appearance of the animals. This is to determine whether the

TABLE V
Level 3 Testing

Pharmacokinetics
- In rats inhaling DME, tissue concentrations rise quickly, reach steady state in 1/2 hr
- When exposure ceases, tissue decline rapid (inverse of uptake)
- No tissue storage
- Biological half-life (blood): α phase, 10 min; β phase, 90 min
- Steady state tissue concentrations proportional to dose from 750–2,000 ppm (range studied)

Developmental toxicity
- Rats, days 6–15, 6 hr/day
- 0, 1,250, 5,000, 20,000, 40,000 ppm
- Maternal response
  Narcosis, 5,000 ppm or greater
  Weight gain suppression, 40,000 ppm (toxicity)
- Fetal response
  No terata
  No increased resorption
  Fetal weight lower, skeletal variations increased 20,000 ppm or greater
- Rats, days 6–15, 6 hr/day
- 0, 20,000, 28,000 ppm
- Maternal response; nothing observed
- Fetal response
  Increased number of supernumerary ribs (skeletal variation)
  No terata
  No increased resorption

Extended subchronic inhalation toxicity

Experiment 1
- Rats, 6 hr/day, 5 days/wk × 13 wk
- 0, 2,000, 10,000, 20,000 ppm
- Increased number of neutrophils, 20,000 ppm, males only
- No other toxicologic changes

Experiment 2
- Rats, 6 hr/day, 5 days/wk × 13 wk
- 0, 5,000, 10,000, 20,000 ppm
- Increased neutrophils, males, all levels (no dose-response)
- Consider 10,000 ppm to be no-observed-effect level in rats

Experiment 3
- Hamsters, 6 hr/day, 5 days/wk × 13 wk
- 0, 5,000, 10,000, 10,000 ppm
- Leukocyte counts (lymphocytes primarily) decreased at 20,000 ppm
- 10,000 ppm, no-observed-effect level

Experiment 4
- Rats, 6 hr/day, 5 days/wk × 30 wk
- 0, 200, 2,000, 20,000 ppm
- SGPT values marginally elevated at 20,000 ppm, 24 weeks, not at 27 weeks (effect in 2,000 ppm, males, suggested)
- Males, 20,000 ppm, elevated liver weights, no pathology
- No-observed effect level 2,000 ppm

fetuses derive from females exposed during gestation are or are not normal.

In the first experiment, animals exposed to DME at 5,000 ppm or more showed some evidence of a narcotic effect, again, somewhat proportional to dose. At a concentration of 40,000 ppm these female animals showed a suppression of body weight gain; this is evidence of toxicity, whereas the narcosis is probably the expected pharmacologic response to this material.

There was no evidence of any teratogenic effect on the fetuses. There was no increase in fetal resorption. What we found at concentrations of 20,000 and 40,000 ppm is some evidence that the fetuses were somewhat smaller, measured in terms of body weight, and that fetal variations such as ossification of the thoracic cavity, the rib bones, and some of the phalangeal bones in the extremities of these animals was somewhat retarded. These we consider as variations reflecting developmental delay rather than a specific effect on the fetus.

A second study conducted in another laboratory used the same animal model, the same dosage, and time—six hours a day—and tested at 20,000 and 28,000 ppm. Those researchers did not find any signs of narcosis in the maternal animals. Maternal body weight gain was normal, so they found no evidence of toxicity. As in our experience, they found a slight increase in the number of extra ribs in the fetuses at both levels tested; the increase was statistically significant, yet there was no dose-response. No gross malformations were noted in these offspring, and the embryo mortality was not reflected by increased resorption.

From these studies, we conclude that the material is not a specific developmental toxin: as expected at doses where one is incurring maternal toxicity, one gets some sign of a response in the fetuses—an increase in fetal variations and some reduction in fetal weight. There are no terata or embryo mortality.

Level 3: Subchronic Inhalation Toxicity. Expanding the data base as the usage patterns expand, we extended the inhalation toxicity experiments to 13 weeks (Table V). We looked at the rat twice and the hamster once, testing at concentrations from zero to 20,000 ppm. These are complete experiments; we look at the total *in vivo* response of the animal: the hematologic profile; liver and kidney function; we look at all of the tissues and organs after the test under the microscope to determine whether there is or is not a treatment-related change.

In the first subchronic inhalation toxicity experiment—at 20,000 ppm, 13 weeks of exposure (Table V), male rats showed a significant increase in the number of neutrophils at the top level tested. All other results were normal and there was no evidence of narcosis in these rats. No tissue changes were seen.

In Experiment 2 (a duplicate of the first) we found marginal increases in neutrophils in males at all three test levels, but no dose-response. The researchers who ran this test compared the neutrophil response to the historical control rather than just this specific control; they concluded that the observation is probably unrelated to treatment and that 10,000 ppm was a no observed-effect level for the rat. They also conducted the study in hamsters (Experiment 3) and reached the same conclusion.

Extending the rat study (Experiment 4), we used the same exposure conditions, for 30 weeks this time, using doses from 200 to 20,000 ppm. At 24 weeks, liver function, as indicated by increases in serum glutamic pyruvic transaminase, appeared to be elevated. The animals had been studied prior to 24 weeks and were again examined at 27 weeks. There was no evidence of change in the white cells, the neutrophils, or the lymphocytes, as seen earlier in the shorter term studies and the liver function tests were normal.

TABLE VI
Level 4 Testing

Lifetime inhalation toxicity
- Rats, 6 hr/day, 5 days/wk × 104 wk
- 0, 2,000, 10,000, 25,000 ppm
- No evidence of an increase in cancer
- No specific tissue damage
- Females only—slight decrease in mean survival at 10,000 and 25,000 ppm
- Hematologic, clinical pathology normal
- No-observed effect level, 2,000 ppm
- Not carcinogenic

At 20,000 ppm in this particular study, liver weights in male rats were marginally elevated but there was no pathology when studied under the microscope. The conclusion of this experiment is that the no-effect level for 30 weeks in the rat is 2,000 ppm.

Level 4. In the lifetime inhalation study (Table VI), the three additional parameters looked at are chronic toxicity, effect upon life span, and carcinogenic potential. In this study, rats again were the animal model, six hours a day, five days a week for the lifetime of the animal, or 104 weeks. We tested concentrations of 2,000, 10,000, and 25,000 ppm. There was no evidence of any increases in cancer in any of the tissues or organs of these animals. Again, there was no specific tissue damage, as indicated by either clinical function studies or morphologic studies of the tissues taken at the end of the study.

Female animals in both the 10,000- and 25,000-ppm groups showed a slight decrease in mean survival time. The decrease is not statistically significant, but it is different than that seen either at the low level or in the control subjects. These animals, for some reason not known to us yet, were somewhat heavier than the corresponding groups. Hematology and clinical pathology studies conducted at three-month intervals during the two years showed no evidence of change.

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ence of pesticides listed on the Groundwater Protection List, the statute directs CDFA to conduct soil and groundwater monitoring in areas where listed pesticides are primarily used.[20] CDFA must commence such monitoring one year after a pesticide is placed on the Groundwater Protection List.[21]

Conclusion

As stated above, the effects of this statute are only beginning to be felt. Its impact on pesticide use in California could be enormous, but will depend largely upon CDFA's enforcement objectives. CDFA's handling of the atrazine and simazine cases will give an indication of its objectives. However, because the act is limited to groundwater contamination from "agricultural use," the nonagricultural pesticide industry, which manufactures pesticides for home, lawn, and garden use and for industrial and institutional use, is not subject to the act.

Addendum

On November 4, 1986, the people of the state of California passed Proposition 65, the Safe Drinking Water and Toxic Enforcement Act of 1986. The act prohibits the release of carcinogens and reproductive toxins into water or onto land in any manner by which the chemical may pass into any source of drinking water. The act also appears to mandate extraordinary label warnings for products containing such chemicals. It is a far-reaching piece of legislation and is likely to significantly affect the operations of business in California. Analysis of this act will be the subject of the next "Letter of the Law." □

References

1. Cal. Food & Agr. Code §13149(b).
2. Cal. Food & Agr. Code §11408. The Pesticide Contamination Prevent Act incorporates this section by reference under §13142(f).
3. Cal. Food & Agr. Code §13149(c).
4. The subcommittee is composed of one representative each from CDFA, the Department of Health Services and the State Water Resources Control Board. Cal. Food & Agr. Code §13150(b).
5. Cal. Food & Agr. Code §13150(a).
6. Cal. Food & Agr. Code §13142(j).
7. Cal. Food & Agr. Code §13150(c).
8. *Id.*
9. Cal. Food & Agr. Code §13150(d).
10. Cal. Food & Agr. Code §13152(b). An exception to automatic cancellation under §13152(b) is preserved for "hardship" pesticides determined not to be carcinogenic, mutagenic, teratogenic, or neurotoxic. The cancellation procedure applicable to such pesticides is not explained by the act.
11. Cal. Food & Agr. Code §13143.
12. *Id.*
13. Cal. Food & Agr. Code §13145.
14. *Id.*
15. 7 U.S.C. §136a.
16. Cal. Food & Agr. Code §13146. CDFA recently proposed regulations to implement §13146 of the Pesticide Contamination Prevention Act. 86 Not. Reg. No. 27-Z (July 4, 1986). Under these regulations, the arbitration procedures established by FIFRA §§3(c)(1)(D) and 3(c)(2)(B) would apply to Cal. Food & Agr. Code §13146.
17. Cal. Food & Agr. Code §13145(d).
18. *Id.*
19. *Id.*
20. Cal. Food & Agr. Code §13148.
21. *Id.*

Dimethyl Ether

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In this study, we've concluded that the no-observed-effect level is 20,000 ppm and stress that we find no evidence of real chronic toxicity. DME is not carcinogenic, and the only finding might be a slight decrease in life span in the female rats.

In summary, DME's combination of high-pressure characteristics, good water solubility, and good solvency cause it to be technically and economically effective as a liquified gas propellant.

Dymel A propellant is now approved for general aerosol use, principally because DME has demonstrated in a variety of toxicologic testing a low order of acute and chronic inhalation toxicity, and that it is not a carcinogen, teratogen, or mutagen. □