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California Air Resources Board

BULLETIN

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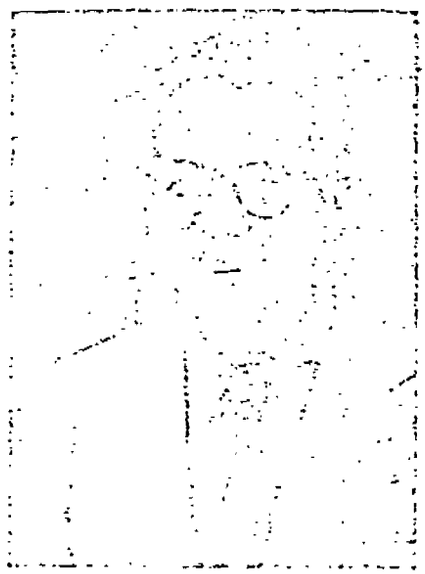
Dr. Gordon appointed ARB member



DR. ALVIN S. GORDON

Governor Edmund G. Brown Jr. on March 8 announced the appointment of Dr. Alvin S. Gordon of Encinitas to the Air Resources Board.

Dr. Gordon succeeds Dr. Robert Sawyer, whose resignation was effective February 29.

Dr. Gordon was a civilian employee of the U.S. Navy at China Lake from 1951 to January, 1970; most recently, he was head of the chemical kinetics branch. Presently Dr. Gordon, who is 61, teaches engineering at the University of California at San Diego.

He has an outstanding background for participation in the work of the Air Resources Board. He received his Ph.D. in physical chemistry from New York University and his B.S. from Polytechnic Institute of Brooklyn. He was a member of the Air Resources Board research screening committee and at China Lake he established research in photochemistry and combustion.

Gradual reduction of lead in gasoline ordered by ARB

The state Air Resources Board voted on February 19 to require oil companies to gradually reduce the amount of lead added to gasoline sold in California.

The action, climaxing a decade of debate on the hazards of airborne lead, was taken after a lengthy hearing at Los Angeles City Hall.

ARB Chairman Tom Quinn said the reduction of lead in gasoline is necessary to protect public health, especially children in heavily populated areas and along freeway routes.

"Many parks, playgrounds and apartment complexes near freeways pose a serious health problem for children because of large concentrations of airborne lead," Quinn said. "There is evidence that children who live in some Southern California areas have 10 times more lead in their blood than is normal."

The unanimous action by the ARB requires large oil refineries to phase down the average lead content of all gasoline produced at each refinery from present levels of around 1.85 grams per gallon to 0.4 by 1980. Lead reductions and effective dates adopted are 1.4 grams per gallon (average) by January 1, 1977, 1.0 by January 1, 1978, 0.7 by January 1, 1979, and 0.4 by January 1, 1980. Since the reductions are based upon a pool average of gasoline produced at the refineries, the companies will be able to offer premium and regular grades for older cars.

Smaller companies, producing less than 20,000 barrels per day, will be required to reduce average lead content to 1.7 grams per gallon by January 1, 1979, and 1.4 by January 1, 1980.

Board member Mary Nichols commented the phase-down is necessary to achieve the State's air quality standard for lead of 1.5 micrograms per cubic meter for a 30-day average. "Above that level, independent medical evidence shows that there is a danger of permanent neurological damage to younger children," she said.

Spokesmen for some major oil companies complained that action could cause economic hardship for them, but Quinn responded that the companies had refused to provide the Air Resources Board with data on costs of removing lead from gasoline.

"The oil companies have grossly exaggerated the problem and made it very difficult for us to do our job," he said. "They have attempted to mislead us and interfere with our efforts to act in the public interests."

While voting in favor of the reduction, Board member Dr. Robert Sawyer said, "I want to go on record as favoring a complete elimination of lead from gasoline in the years ahead."

Under the new controls, the ARB estimates the state will be able to achieve lead standards 99 percent of the time in the South Coast Air Basin, with the exception of the Lennox area which has recorded exceptionally high lead levels.

March 30-31 meetings set for San Francisco

California Air Resources Board will meet March 30 and 31 in San Francisco.

Included on the agenda of the March 30 meeting will be a continued public hearing on procedures for certification of gasoline vapor recovery systems at service stations, public hearing to consider adoption of ambient air quality standards applicable to the Lake Tahoe Air Basin only, and a review of the enforcement practices of the Bay Area Air Pollution Control District.

Two ambient air quality standards different from the rest of the State are being considered for Lake Tahoe: visibility reducing particles and carbon monoxide.

The San Francisco meetings will be

(Continued on page 3)

D. Reich TCE - Mutagen
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Br. J. Anaesth. (1979), 51, 417

MUTAGENICITY OF INHALATION ANAESTHETICS: TRICHLOROETHYLENE, DIVINYL ETHER, NITROUS OXIDE AND CYCLOPROPANE*

J. M. Baden, M. Kelley, R. I. Mazze and V. F. Simmon

SUMMARY

The mutagenic potential of trichloroethylene, divinyl ether, nitrous oxide and cyclopropane was assessed *in vitro* by microbial assay employing two histidine-dependent strains of *Salmonella typhimurium*, TA1535 and TA100. Anaesthetic agents in various concentrations were incubated with bacteria in the presence or absence of an enzyme system prepared from enzyme-induced rat liver. Nitrous oxide and cyclopropane were not mutagenic, whereas divinyl ether gave a strongly positive response. Results for trichloroethylene were equivocal. These and previous studies with the salmonella system, together with mutagenicity studies using different test systems, indicate that modern inhalation anaesthetic agents are unlikely to be mutagenic.

Among the suggested hazards of operating room contamination by anaesthetic gases is an increased frequency of malignancies in female anaesthetists (Cohen et al., 1974). It is therefore appropriate to screen the anaesthetic agents for possible carcinogenic potential. The salmonella–microsome test (Ames, McCann and Yamasaki, 1975) is an assay that has proved useful in the detection of chemical carcinogens as mutagens. We used this procedure to test halothane, and four halogenated ethers—methoxyflurane, isoflurane, enflurane and fluroxene (Baden et al., 1976, 1977)—for mutagenic activity; only fluroxene (2,2,2-trifluoroethyl vinyl ether) was mutagenic (Baden et al., 1978). In the present study we have tested the volatile anaesthetics trichloroethylene and divinyl ether and the gaseous anaesthetics, nitrous oxide and cyclopropane, to determine their mutagenic potential.

METHODS

The methods and materials used for testing anaesthetic agents in the salmonella assay system have been reported in detail elsewhere (Baden et al., 1976) and are summarized below. The anaesthetics tested were commercially available preparations. Trichloroethylene and divinyl ether were greater than 99.5% pure as assayed by gas chromatography.

Bacterial preparation

Two histidine-dependent strains of *Salmonella typhimurium*, TA1535 and TA100, were used (Ames, McCann and Yamasaki, 1975). For each experiment, inocula from stock cultures grown overnight at 37 °C were used in a nutrient broth.

Metabolic system

A mammalian metabolic activation system, S-9 mix, was prepared from the livers of male Sprague-Dawley rats sacrificed 5 days after i.p. injection of 500 mg kg^{-1} of Aroclor 1254, a polychlorinated biphenyl. Aroclor is a potent inducer of the mixed-function oxidase system. Each assay was conducted both with and without the metabolic activation system.

Desiccator incubation experiments

Bacteria-seeded glucose-minimal plates with or without S-9 mix were placed in 9-litre, air-tight desiccator jars. Liquid or gas volumes of anaesthetics were added to the desiccators to give desired concentrations, which were verified at the beginning and end of exposure using an infra-red gas analyser. The concentrations, ±10% tolerance, ranged from 0.1 to

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30% for divinyl ether, 0.1 to 10% for trichloroethylene and 1 to 81% for nitrous oxide and cyclopropane. The concentrations did not change significantly with time. Vinylidene chloride 3% was used as a positive control. The bacterial plates were exposed to the test compound in sealed desiccator jars for 8 h at 37 °C then the plates were removed from the desiccators and incubated for a further 40 h at 37 °C. Colonies on each plate were counted. Triplicate plates were prepared at each test concentration, and each experiment was performed at least twice.

Liquid incubation experiments

Bacterial cells, with or without S-9 mix, and various amounts of test chemical were added to sterile, 15-ml stoppered tubes and rotated on a wheel for 2 h at 37 °C. The air phase concentrations for a particular anaesthetic, in the air spaces above the reaction mixtures, were the same as those used in the desiccator experiments. Concentrations were measured at the beginning and end of exposure, using an infra-red gas analyser, and were found to vary by not more than 10% with time. Aliquots of the test sample were added to tubes of top agar, mixed and poured onto glucose-minimal medium plates. 2-Anthramine 2.5 μg per plate was used as a positive control. The plates were incubated for 2 days at 37 °C in air and colonies were counted. Triplicate plates were prepared at each test concentration, and each experiment was performed at least twice.

Chemicals

The source and purity of chemicals were as follows: trichloroethylene, Aldrich Chemical Co. (Milwaukee, Wisconsin), 99%—contains no trace of 1-2 epoxybutane or epichlorohydrin; divinyl ether, Marshallton Chem. Co. (Winston, Salem, N.C.) 99.9%; nitrous oxide, Liquid Air, Inc. (San Francisco, California), 99%; cyclopropane, Liquid Carbonic (San Carlos, California), 99.9%.

Analysis of data

The numbers of revertant colonies on treated plates were compared with the numbers of spontaneous revertant colonies on plates exposed to room air. Statistical analysis was by t test; $P<0.05$ was considered significant.

RESULTS

Nitrous oxide and cycloprane did not increase the number of revertants in strain TA100 when assayed either in desiccators or in liquid incubation (table I). Similar negative results were obtained with strain TA1535. In contrast, plates exposed to the positive controls, vinylidene chloride or 2-anthramine showed 2.4–14.4-fold increases in the number of revertant colonies.

Divinyl ether was mutagenic to strains TA100 and TA1535 in desiccator and liquid incubation. In desiccators, a dose-dependent increase in revertants occurred in the absence of metabolic system at vapour

TABLE I. *Mutagenicity*, Salmonella typhimurium *strain TA*100 (*mean number of revertant colonies per plate* ± *SEM*, $n = 6$)

Concn (vol %)		Nitrous oxide		Cyclopropane	
		Without S-9	With S-9	Without S-9	With S-9
Desiccator	0	144 ± 16	167 ± 8	167 ± 12	183 ± 16
	1	167 ± 3	177 ± 8	144 ± 11	157 ± 12
	3	158 ± 14	178 ± 18	149 ± 10	157 ± 8
	9	153 ± 7	147 ± 7	166 ± 10	153 ± 10
	27	162 ± 17	155 ± 9	168 ± 8	181 ± 16
	81	144 ± 20	160 ± 10	125 ± 14	122 ± 9
	Vinylidene chloride 3%	669 ± 63	403 ± 34	886 ± 56	604 ± 37
Liquid	0	115 ± 10	117 ± 10	92 ± 1	90 ± 5
	1	104 ± 10	98 ± 11	91 ± 5	84 ± 6
	3	89 ± 6	103 ± 15	91 ± 7	86 ± 3
	9	83 ± 5	110 ± 4	86 ± 2	89 ± 7
	27	98 ± 8	101 ± 8	96 ± 6	86 ± 6
	81	92 ± 9	99 ± 11	91 ± 5	92 ± 7
	2-anthramine 2.5 μg/plate	107 ± 10	1122 ± 93	117 ± 8	1299 ± 166

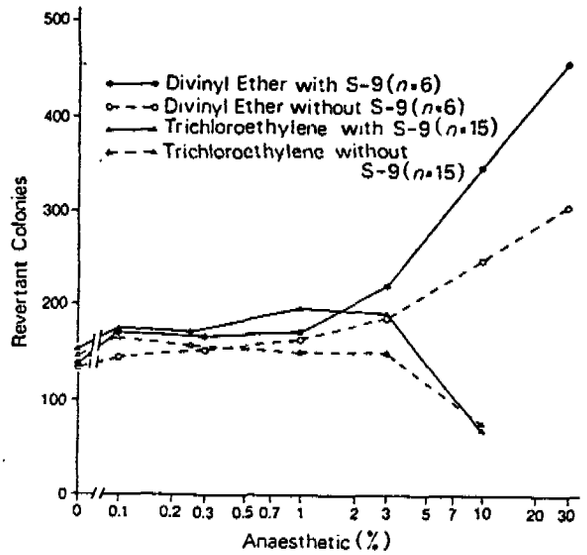


FIG. 1. Revertant colonies of *Salmonella typhimurium*, strain TA100, after desiccator incubation with divinyl ether and trichloroethylene. Divinyl ether produced a dose-dependent increase in the number of revertant colonies at vapour concentrations greater than 1%; the maximum increase above background was 2.5–3.5-fold when S-9 was added. Trichloroethylene produced a 30% increase in the number of revertant colonies only in the presence of S-9.

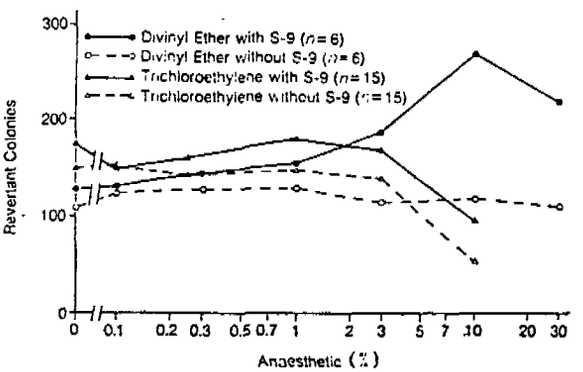


FIG. 2. Revertant colonies of *Salmonella typhimurium*, strain TA100, after liqiud incubation with divinyl ether and trichloroethylene. Divinyl ether produced a dose-dependent increase in the number of revertant colonies in vapour concentrations greater than 1%. The increase was seen only in the presence of S-9 and reached a maximum of 2.5-fold above background. Trichloroethylene did not increase the number of revertants above background.

concentrations greater than 1%; when the metabolic system was added, there was an increase above background of 8–11-fold for TA1535 and 2.5–3.5-fold for TA100. In liquid incubation, a mutagenic response occurred only in the presence of the S-9 mix. Results are shown for strain TA100 in figures 1 and 2.

Trichloroethylene did not increase the number of revertants in strain TA1535 or when assayed by liquid incubation. In desiccators, however, with the metabolic system present, the mean number of revertants of TA100 was increased approximately 30% above background (149 ± 12, $n = 15$) at 1% (196 ± 13, $n = 15$) and 3% (194 ± 6, $n = 15$) vapour concentrations (fig. 1.). These increases were statistically significant ($P < 0.01$).

DISCUSSION

In the present study, the most significant findings occurred with divinyl ether which was mutagenic to both strains of salmonella. Both the gases, nitrous oxide and cyclopropane, were not mutagenic while the results of the trichloroethylene tests were equivocal. In similar studies of halothane, enflurane, methoxyflurane and fluroxene, only fluroxene (2,2,2-trifluoroethyl vinyl ether) was mutagenic (Baden et al., 1978). Fluroxene, however, gave positive results only in liquid incubation, whereas divinyl ether was mutagenic in both liquid and desiccator incubation. In the desiccator assay, divinyl ether was mutagenic without the metabolic systems, although its activity was enhanced when metabolizing enzymes were present. This finding indicates that both divinyl ether and one or more of its metabolic products are mutagenic. In the past it has been assumed that the toxic effects of anaesthetic agents were produced only by reactive anaesthetic metabolites. Either divinyl ether was directly mutagenic or salmonella metabolized the anaesthetic to a mutagen. Many other compounds containing the vinyl moiety are mutagens (McCann et al., 1975) presumably because this group is highly reactive and can be oxidized to an epoxide (Bartsch et al., 1975).

The findings with trichloroethylene show that it is either not, or only weakly, mutagenic. A 30% increase in revertant colonies occurred reproducibly at 1 and 3% vapour concentrations in desiccator assays with strain TA100. Some authors (Chiu et al., 1978) would not regard this as a positive result, believing that at least a 100% increase in the number of revertant colonies is needed before mutagenicity is is established. Waskell (1978), who has also tested trichloroethylene in the salmonella system, concluded that it was not mutagenic. However, the test conditions used were not the same as in the present study. On the other hand, Simmon, Kauhanen and Tardiff (1977), in a study using metabolic activation

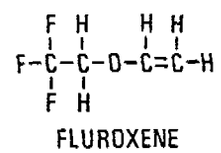
systems prepared from both mice and rats, concluded that trichloroethylene was a weak mutagen. This conclusion is supported by the high frequency of hepatocellular carcinoma in B6C3F1 mice treated with commercial trichloroethylene (National Cancer Institute, 1976). Unfortunately, the test samples used in the mouse studies were stabilized with carcinogenic epoxides (Henschler et al., 1977) and were given in large quantities into the stomach. Thus the mutagenic and carcinogenic potential of trichloroethylene remains uncertain.

TABLE II. *Mutagenicity tests: A = salmonella—Ames test; B = sister chromatid exchange; C = fibroblast–8-azagnanine*

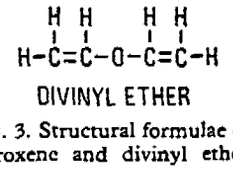
Agents	Positive	Negative
Halothane	—	A, B, C
Nitrous oxide	—	A, B, C
Chloroform	—	A, B, C
Enflurane	—	A, B, C
Methoxyflurane	—	A, B
Isoflurane	—	A, B
Cyclopropane	—	A, B
Diethyl ether	—	A, B
Trichloroethylene	A?	A, B
Fluroxene	A, B	—
Divinyl ether	A, B	—
Ethylvinyl ether	B	—

Two other mutagenicity test systems have been used recently to test anaesthetics and in general have confirmed the findings of studies in the salmonella–microsome assay (table II). Sturrock (1977) reported negative results for halothane, chloroform, nitrous oxide and enflurane, using the cultured Chinese hamster fibroblast–8-azaguanine system. The effect of anaesthetics on sister chromatid exchange in Chinese hamster ovary cells also has been measured (Stevens et al., 1977). Nitrous oxide, diethyl ether, trichloroethylene, halothane, enflurane, isoflurane, methoxyflurane and chloroform had no effect, whereas divinyl ether, vinyl ethyl ether and fluoroxene gave positive results.

The salmonella–microsome assay has proved useful in the detection of carcinogens as mutagens. Approximately 85% of known animal and human carcinogens examined in this system were mutagenic and nearly all mutagens are carcinogenic (Ames, McCann and Yamasaki, 1975). Our results with the salmonella assay have been supported by data from the Chinese hamster fibroblast–8-azaguanine and sister chromatid exchange systems. Based on all the evidence,



FLUROXENE



DIVINYL ETHER

FIG. 3. Structural formulae of fluroxene and divinyl ether.

we conclude that fluroxene and divinyl ether containing the vinyl moiety (fig. 3) are potential carcinogens. These studies also suggest that the modern inhalation anaesthetic agents, nitrous oxide, halothane, enflurane, cyclopropane and methoxyflurane, are not carcinogenic.

ACKNOWLEDGEMENT

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MUTAGENICITE DES ANESTHESIANTS PAR INHALATION: TRICHLOROETHYLENE, ETHER DIVINYLE, PROTOXYDE D'AZOTE ET CYCLOPROPANE

RESUME

On a évalué *in vitro* le potentiel mutagénique du trichloroéthyléne, de l'éther divinyle, du protoxyde d'azote et du cyclopropane par essai de conformité microbien à l'aide de deux souches de *Salmonella typhimurium* TA1535 et TA100, dépendant d'histidine. Des agents anesthésiants, en diverses concentrations, ont été mis à incuber avec des bactéries, en présence ou en l'absence d'un système d'enzyme préparé à partir de foie de rat traité aux enzymes inductibles. Le protoxyde d'azote et le cyclopropane n'ont pas été mutagéniques, alors que l'éther divinyle a donné une forte réaction positive. Les résultats obtenus avec le trichloroéthylène ont été équivoques. Ces études, ainsi que les précédentes, effectuées avec le système de salmonella, de même que les études de mutagénicité effectuées à l'aide de différents systèmes d'essais, indiquent que les anesthésiants modernes par inhalation ne sont de toute vraisemblance pas mutagéniques.

DIE MUTATIONSFÄHIGKEIT DER INHALATIONSNARKOSE: TRICHLORÄTHYLEN, DIVINYLÄTHER, LACHGAS UND ZYKLOPROPAN

ZUSAMMENFASSUNG

Es wurde das mutagene Potential von Trichloräthylen, Divinyläther, Lachgas und Zyklopropan *in vitro* mittels Mikrobenanalyse beurteilt, in der zwei histidin-bezogene Stämme von *Salmonella typhimurium*, TA1535 und TA100, verwendet wurden. Narkosemittel in verschiedenen Konzentrationen wurden mit Bakterien in der Gegenwart oder Abwesenheit eines Enzymsystems inkubiert, das von, in Rattenleber herbeigeführten Enzymen hergestellt wurde. Lachgas und Zyklopropan waren nicht mutagen, wohingegen Divinyläther eine stark positive Reaktion ergab. Für Trichloräthylen waren die Ergebnisse zweideutig. Diese und vorangehende Studien mit dem Salmonella-System deuten zusammen mit Mutationsfähigkeitsuntersuchungen, die andere Testsysteme verwenden, darauf hin, dass es unwahrscheinlich ist, dass moderne Inhalationsnarkosemittel mutagen sind.

MUTAGENICIDAD DE ANESTESIAS POR INHALACION: TRICLOROETILENO, ETER DIVINILICO, OXIDO NITROSO Y CICLOPROPANO

SUMARIO

Se evaluó el potencial mutagénico de trocloroetileno, éter divinílico, óxido nitroso y ciclopropano *in vitro* mediante evaluación microbial, empleando dos cepas de *Salmonella typhimurium* dependientes de histidina, TA1535 y TA100. Se incubaron diversas concentraciones de agents anestésicos con bacteria en la presencia o ausencia de un sistema de enzimas preparado con hígado de rata inducido con enzimas. El óxido nitroso y el ciclopropano no fureon mutagénicos, mientras que el éter divinílico dio una respuesta positiva fuerte. Los resultados del tricloroetileno fueron ambiguos. Tanto éstos como los estudios anteriores realixados con el sistema de salmonella, juntamente con estudios de mutagenicidad que se valieron de pruebas diferentes, indican que es poco probable que los agentes anestésicos modernos por inhalación sean mutagénicos.