HSIA

FILE → 1,1,1-Trichloroethane K-1716-(100)

Vinyl Chloride K-1711-(100) [1983]

Trichloroethylene K-2520-(100)

Perchloroethylene K-2521-(100)

Butylene Oxide K-10841-(100)

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February 14, 1983

TO: HSIA HEALTH ASSESSMENT GROUP

RE: PPG GENOTOXICITY STUDY

Gentlemen:

Enclosed, for your information, find the final report of the study run for PPG by G. Williams covering the following:

1,1,1-Trichloroethane
1,1,2-Trichloroethane
Tetrachloroethylene

Alan W. Rautio
Executive Director

☆ PPG ☆
Study

PPG INDUSTRIES, INC.

FINAL REPORT

JANUARY 1983

EVALUATION OF SEVERAL HALOGENATED ETHANE AND ETHYLENE COMPOUNDS FOR GENOTOXICITY

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Technical Report

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I. Objectives:

The objectives of this study are as follows:

1. To develop modified methods of the Ames Salmonella/microsome mutagenesis and the Williams Hepatocyte Primary Culture/DNA repair assays which are suitable for detecting genotoxicity of gaseous compounds.
2. Using newly developed methods, to evaluate the genotoxicity of three halogenated ethane and ethylene products with vinyl chloride as a positive control.

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II. Materials and Methods

A. Vapor Equivalent:

Calculation of liquid volume required to produce a desired concentration of the compound in the gas phase: The vapor equivalent of liquids can be calculated by the following equation:

$$V = C \times Vl \times \frac{MW}{22.4} \times \frac{273}{T} \times \frac{P}{760} \times \frac{1}{d} \times 10^{-2}$$

Where Vl: Chamber volume in liters
MW: Molecular weight of a test compound
T: Absolute temperature in chamber
P: Atmospheric pressure in mm Hg
d: Density (gram per ml.) of liquid test compound.
V: Volume of the test compound to be used (in ml)
C: Target concentration (%) of test compound in vapor in chamber.

Since the internal dimension of the chamber is 22.2x28.8x21.5 cm, the volume is 13.68 l. Since the exposures are performed at atmospheric pressure P=760mm Hg and variations by temperature effects are small and are within sampling error range, the following is derived.

$$V = \frac{C \times 13.68 \times MW}{d \times 10^2 \times 22.4}$$

Examples of Calculated Liquid Volume Used in the Chamber:

Chemical Vapor Target Conc (%)	Chemical Liquid Volume (ml)			
	Vinyl Chloride	1,1,1-Tri-chloroethane	1,1,2-Tri-chloroethylene	Tetrachloro-ethylene
5.0	2.1	3.7	2.7	3.1

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B. Test Compounds

The test compounds examined in this study were vinyl chloride; 1,1,2-trichloroethylene, non-stabilized and stabilized; 1,1,1-trichloroethane, low-stabilized and stabilized; tetrachloroethylene, non-stabilized, stabilized, and with high purity (Table I). All test compounds were obtained from PPG Industries, Lake Charles plant. Analyses of purity were performed by the Barberton Technical Center. All test compounds were stored at -20°C at which vinyl chloride stayed in a liquid state.

C. Salmonella/microsome mutagenesis assay

1. Mutagenesis Assay: Modified exposure method:

The assay developed by Ames (1) was modified for the study of gas phase compounds.

The bacterial cultures were exposed in an aluminum chamber with internal dimensions of 22.2 cm X 28.8 cm x 21.5 cm and a plexiglass front door. Because of the size of the chamber and the possibility of compounds reacting with plastics, 60 mm glass petri dishes were substituted in place of conventional 100 mm plastic petri dishes. The internal surface area of the plexiglass door was covered with aluminum foil. Each petri dish was layered with 10 ml of minimum agar. The top agar mixture was prepared according to the conventional method by mixing 0.1 ml of the tester strain, 0.1 ml of phosphate buffer in place of a test compound, 0.5 ml of S-9 mix or phosphate buffer for activation-free exposures and 2 ml of molten top agar (total of 2.7ml), and 0.9 ml of this mixture was plated on top of the base agar layer

in triplicate petri dishes. Two hours was allowed for initiation of bacterial growth in soft agar.

The exposure to gas phase of vinyl chloride (VC) was performed as follows: calibrated volumes that would produce the desired gas-phase concentrations upon evaporation were poured into a deep glass petri dish containing a magnetic bar. The dish containing liquid phase VC was immediately placed at the bottom of the exposure chamber in which uncovered bacterial culture dishes were in place. The chamber was tightly closed and placed on a magnetic stirrer at room temperature for 20 min to allow the evaporation of VC in the chamber. Then the chamber was transferred to a 37°C incubator and the exposure continued for 18 hrs. with continuous stirring to dissipate the gas evenly inside of the chamber. At the end of the exposure period, the gas phase compound was released from the chamber through a rear valve inside a hood with external exhaust. The bacterial cultures exposed to gas were removed from the chamber, covered with lids, and incubated for another 30-54 hrs. in the absence of the test compound in an incubator. After a total of 48-72 hrs. incubation, the number of revertant colonies was counted and the average of triplicate plates was multiplied by 3 to adjust to conventional assay conditions. The control plates used to score spontaneous revertants were prepared in similar manner and incubated in a regular incubator in the absence of test compounds.

2. Cytotoxicity Test:

In order to determine the cytocidal effects of gaseous compounds, cytotoxicity tests were performed simultaneously with the

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mutagenicity studies. Nutrient agar plates overlayered with top agar containing a tester strain that was diluted to the concentrations which yield a countable number of colonies and S-9 mix or phosphate buffer were placed in the chamber simultaneously with mutagenicity assay plates. Non-exposed control plates containing tester strains with or without S-9 mix were incubated in a 37°C incubator. The cytotoxicity of the test compounds is expressed as the percentage reduction in the colony number.

3. Interpretation of results:

Revertant colonies on the plates were counted after total of 48 - 72 hrs. incubation at 37°C. The plates which yielded 100 - 150 revertant colonies per 60mm plate were used for numerical expression of the mutagenicity of a compound in this assay. The average number of induced revertants for 3 plates after subtracting the number of spontaneous revertants is expressed as the number of revertants per dose per plate.

Cytotoxicity of the compound at each dose is expressed as the reduction in numbers of colonies relative to that of the non-exposed set. The compound is reported negative in this assay if the number of induced revertants did not exceed twice the spontaneous revertants (1) at the highest doses that did not exceed 20% toxicity.

D. Hepatocyte Primary Culture (HPC)/DNA repair assay

1. Initiation of Hepatocyte Cultures:

Rat hepatocyte primary cultures (HPC's) were initiated according to the modified procedures of Williams (2).

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Adult male Fischer strain 344 rats were anesthetized with 50 mg/kg body weight Nembutal Sodium Solution (Abbott Laboratories). Perfusion of liver was performed with a peristalic pump which was sterilized by circulation of 70 % ethanol followed by sterile water.

A ventral midline incision was made from the xiphisternum to the pubic bone and the liver was exposed. A 21 gauge butterfly needle was inserted in the portal vein and clamped in place with a serrafine forceps. Perfusion was then commenced with 37°C sterile Solution I which consisted of 0.5 mM ethylene glycol-bis(B-aminoethyl ether) N-N'tetracetic acid (EGTA) (Sigma) in Ca++, Mg++ free Hanks' balanced salt solution buffered with 10mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (Hepes) (Calbiochem), pH Adjusted to 7.35 with 1N NaOH. A perfusion rate of 8 ml/min. is maintained for 1.5 min.

Immediately following the start of perfusion with Solution I, ligation of the infrahepatic inferior vena cava was completed and the vein was severed distally to permit the perfusate to run to waste. While this first perfusion was being performed, the thoracic inferior vena cava was cannulated by puncturing the right atrium and the perfusate was collected via this return cannula. At this point, the proximal segment of the subhepatic inferior vena cava was clamped to close the system and the pump speed was then increased to 40 ml/min for 2.5 min.

Completion of perfusion with Solution I was followed by perfusion with 250 ml of a sterile Solution II, containing 100 unit/ml type 1 collagenase (Sigma) in Williams medium E (WME)

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buffered with 10 mM Hepes, pH adjusted to 7.35. This solution at 37°C was perfused for 12 min at 20 ml/min without recirculation of the return perfusate. The liver was covered with sterile gauze and a 40W bulb positioned 6 cm above the liver for warming.

Following perfusion, the liver was removed to a sterile petri-dish containing warm WME and, in a sterile hood, was trimmed of extraneous fat and connective tissues. The liver was then removed to a second petri-dish containing 50 ml of Solution II. The portis hepatis was grasped with forceps and the capsule of the liver was opened at numerous points on the inferior surface with small scissors and removed. Cells were detached by gentle combing with a stainless steel comb, leaving a fibrous plug of hepatic connective tissue to be discarded. Using a wide bore pipet, 25 ml aliquots of the suspended hepatocytes were pipetted into 50 ml centrifuge tubes, and the volume brought to 50 ml per tube with WME supplemented with calf serum at 10% (WME-CS_{10}). Cells were sedimented at 50xG for 25 min, suspended in WME-CS_{10} and gently mixed by inverting each tube several times. A 20-fold dilution of this cell suspension is prepared and 0.5 ml of the diluted hepatocytes was added to 0.1 ml of 0.4% trypan blue stain (Gibco) for determination of viability using a hemocytometer.

The perfusions performed in this study yielded 0.8-2.0x10^8 cells per 100g body weight and only those preparations with a cell viability higher than 90% were used.

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2. HPC/DNA repair assay:

a) Modified long term vapor phase exposure: The HPC/DNA repair assay developed by Williams (3) and modified (4) was further adapted to the present studies as follows: One million cells were seeded on 4.9 sq cm glass coverslips placed in 60 mm glass petri dishes with 4 ml of WME-CS_{10}. After 3 hrs of attachment, the cells were washed with L-15 medium (phosphate buffered for use in non CO_2 incubator) containing a total of 40uCi [^{3}H]TdR ([^{3}H]TdR L-15) for incorporation during DNA repair synthesis. The dishes were placed in the chamber and the test concentrations of liquid phase compounds were introduced in the same manner as described in C.1. After 18 hrs exposure to test compounds, coverslips were processed according to the conventional method (3) for preparing autoradiographs.

b) Modified short term vapor phase exposure: In order to assess repair of damaged DNA at reduced cytotoxic levels of test compounds, the exposure time was shortened. The cell preparation and exposure method remained the same as described in 2.a) except that exposures were reduced to 3 hrs. After this, exposed cultures were removed from the chamber and washed with two changes of serum free Leibovich's L-15 medium. Then the cultures were fed with 4ml of [^{3}H]TdR L-15 and incubated at 37°C for another 15 hrs to allow DNA repair synthesis.

3. Interpretation of results:

The scoring method routinely used was designed to avoid false positives by choosing the highest cytoplasmic count of each cell as a background and by adjusting number of cells that were

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scored to meet statistical significance, depending on the nuclear/cytoplasmic grain count ratio.

Only those cells which were viable at the time of fixation, indicated by swollen nuclei (as a result of incubation in the hypotonic sodium citrate), and those evenly coated with emulsion were scored. Between 5 to 20 cells randomly selected from each quadrant of the coverslip were counted. The number of cells to be scored depends on the nuclear/cytoplasmic ratio of grains required to meet statistical significance. Counts were obtained for each nucleus; background was determined by counting 3 nuclear sized areas adjacent to the nucleus. Net nuclear grain counts were calculated by subtracting the highest cytoplasmic count from the nuclear count.

Results are reported as the mean ± standard deviation of net grain counts. The test compound is reported positive when the minimum net grain count exceeded 5 per nuclei and was consistantly above controls in 2 experiments.

Cytotoxicity of the test compound was identified by the absence of S-phase cells in the autoradiograph and by general morphology and scored according to the scale 1 + (25% of total cells showing toxic effects), 2 + (50%), 3 + (75%) and 4 + (nearly 100%). When there were not enough cells with intact nuclei and cytoplasma because of extensive cytotoxicity, the sample was designated as "not countable."

III. Results

A. Concentration of gas phase compounds

Gas chromatographic analysis of gas phase VC in the chamber (Table II) verified the reliability of the gas equivalent liquid calibration, the transfer technique of liquid into the chamber and the seal of chamber.

Furthermore, in order to detect any possible reaction of 1,1,1-trichloroethane or 1,1,2-trichloroethylene with the aluminum of the exposure chamber, the concentrations of these gases in air in chamber were analyzed. As shown in Table III, gas chromatographic analysis of these gases at 17 hours were within the range of sampling error indicating retention of these gases in the chamber and no reaction with aluminum.

B. Salmonella/microsome mutagenesis assay

As shown in Tables IV-a and Fig. 1, vinyl chloride (VC) induced reversion mutation at the histidine locus of the strains TA100 and TA1535 in the presence of the activation system in a dose related manner without cytotoxicity. In the absence of the activation system, the recovery of revertants of TA100 did not exceed 2-fold the background, whereas with TA1535, mutations were induced. The greater increase in the number of revertants in the presence of the Aroclor-induced rat S-9 mix indicates metabolic activation of this compound. However, VC did not induce revertants in the other strains examined (TA98, TA1538 and TA1537) even in the presence of an activation system (Table V) indicating that it is a base-pair mutagen.

All test compounds were studied using all 5 tester strains,

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TA100, TA98, TA1535, TA1538 and TA 1537. The only tester strains in which a near two-fold increase in revertants occurred were TA100 and TA1535; therefore the assays were only repeated on these two tester strains.

1,1,1-trichloroethane with low concentration of stabilizer produced no increase in revertants in either TA100 or TA1535 at up to 7.5% vapor concentrations which killed more than 90% of the organisms. At 10%, which had greater than 95% toxicity, revertants were recovered at above 2-fold increases in strains TA100 and TA1538. The addition of an S-9 preparation had no effect.

1,1,1-trichloroethane with stabilizer induced revertant colonies of TA100 at the concentrations of 5% and 7.5% only in the presence of activation systems (2.6-fold and 2.9-fold increases respectively). These mutagenic effects occured at levels of toxicity of 30% or less. The tester strain TA1535 was more sensitive to this material by demonstrating significantly increased revertant induction even at the concentration of 2.5% (a 12.4-fold increase in the absence of S-9 mix and an 8.3-fold increase in the presence of S-9 mix)(Table IV-a).

Non-stabilized 1,1,2-trichloroethylene showed extensive cytotoxicity without revertant induction at any dose in either TA100 or TA1535.

1,1,2-trichloroethylene with stabilizer killed both tester strains completely at the concentrations above 7.5%, but induced significantly high number of revertant colonies at 5% both in TA100 (greater than 4-fold increase) and TA1535 (greater than 37-fold increase) regardless of the presence of S-9 mix. At this

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dose, toxicity exceeded 95%. With TA1535, revertant induction was also observed at the doses of 2.5% (4-fold increase) and 1% (2-fold increase) even in the absence of S-9 mix. Toxicity was moderate at these doses (Table IV-a).

Non-stabilized tetrachloroethylene was cytotoxic at the doses of 5% and greater. An increased recovery of revertant colonies (5-fold increase) occurred only in TA1535 and at greater than 99% toxicity (Table IV-b). The presence of an S-9 fraction made no contribution.

Tetrachloroethylene with stabilizer showed total cell killing at concentrations of 5% and greater. At 2.5%, which was greater than 97% toxic, induced revertant colonies were observed in both tester strains TA100 (2-fold increase) and TA1535 (10-fold increase). In both strains, the presence of activation systems did not make a significant contribution.

Tetrachloroethylene of high purity demonstrated total cell killing effects at a concentration of 2.5%, but did not induce revertants in TA100 or TA1535 at lower doses either in the presence or absence of S-9.

C. HPC/DNA repair assay

As shown in Table VI exposure of hepatocytes to vinyl chloride in the gas phase induced unscheduled DNA synthesis indicating the production of DNA damage to the hepatocytes. The induction of DNA repair by vinyl chloride was observed at 5% without cell killing effect and was dose related within the doses we examined, 5%, 2.5%, 0%, by modified exposure method (Table VI).

Of six test materials examined in this assay, neither

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1,1,1-trichloroethane with low or regular concentrations of stabilizer, nor 1,1,2-trichloroethylene without or with stabilizer induced DNA repair even at doses that exhibited extensive cell killing either by the modified gas phase exposure (Table VI) or by the conventional liquid phase exposure method (Tables VII-a and VII-b). Tetrachloroethylene in the non-stabilized form elicited DNA repair synthesis when hepatocytes were exposed to 0.1% of this compound by the modified method and in the stabilized material at 0.1% (Table VI). Decrease in time of exposure to these compounds did not reduce their cytotoxic effect on hepatocytes nor did it induce DNA repair (Tables VI, VII-a and VII-b).

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IV. DISCUSSION

In this study, the genotoxicity of one chlorinated ethane and two chlorinated ethylene compounds was compared to that of vinyl chloride by measurement of mutation at the histidine locus in Salmonella typhimurium and induction of unscheduled DNA synthesis in hepatocytes. Compounds were tested by vapor phase exposure in both tests as well as liquid phase exposure in the HPC/DNA repair test.

With respect to Salmonella/microsome mutagenicity test, mutagenic activity of vinyl chloride, a positive control used in this study, was in accord with a previous report (8).

Exposure to 1,1,1-trichloroethane with stabilizer increased the recovery of revertants of TA1535 and TA100 with little influence of the S-9 mix (Table IV-a, Fig.3); however, 1,1,1-trichloroethane with low concentrations of stabilizer did not induce revertants (Table IV-a, Fig.2) within the dose range of 1,1,1-trichloroethane with stabilizer that produced an increased revertant recovery. This suggests that the compound used to stabilize trichloroethane may be responsible for the induction of revertant colonies. The pH of 1,1,1-trichloroethane was neutral at the time of testing and therefore acidic decomposition during storage did not occur.

A potential role of the stabilizer in the induction of reverse mutation was also seen in the case of 1,1,2-trichloroethylene (TCE, Figs. 4 and 5). Trichloroethylene without stabilizer did not induce reverse mutations even at a dose that killed 97% of the cells (Table IV-a), whereas TCE with stabilizer

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induced both high cytotoxicity (95%), and revertant colonies (above 4-fold in TA100 and 37-fold in TA98; Table IV-a). These data suggest that the induction of revertant colonies may be attributable to the stabilizer for this solvent but that cytotoxicity may be an effect of TCE itself.

At concentrations of tetrachloroethylene which were highly cytotoxic (97%), a greater reversion response was produced by the stabilized material than the non-stabilized, while no enhancement of revertants were seen following exposure to high purity tetrachloroethylene.

The induction of revertants by the stabilized products of these halogenated ethane and ethylene compounds demonstrates the need for consideration of the role of stabilizers in the mutagenicity of test materials that represent mixtures. The data are consistent with stabilizer involvement since there was no enhancement of revertants associated with the presence of the S-9 fraction. Typical stabilizers, including those found in these solvents, are biologically active without enzymatic activation. Additionally, since the chlorinated ethane/ethylenes did not exhibit greater reversion activity in the presence of S-9 (as did vinyl chloride, a true mutagen), this increased response at high cytotoxic levels does not likely reflect true mutagenic activity. A previous report by Henschler et al. (9) on technical trichloroethylene also demonstrated the important role of stabilizers. They found that epichlorohydrin and 1,2-epoxybutane, which were present as stabilizers, were highly mutagenic against Salmonella tester strains and suggested that they could be responsible for carcinogenic effects in mice (9).

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The other important factor in the interpretation of results is the degree of cytotoxicity. The increase in revertant colonies at the doses which kill more than 95% of the cells (Tables IV-a and IV-b) is of unknown mutagenic significance because mechanisms other than those normally thought to be responsible for causing bacterial mutations (DNA-chemical interactions) may be operative. The different results in the mutagenesis assay between the bonafide mutagen vinyl chloride (high reversion rate at low cytotoxicity, dose-response relationship) and the three solvents (an increase in recovery of revertants only at high cytotoxicity and a lack of dose-responsiveness) further support the interpretation that the latter materials possess no mutagenic activity.

In testing the halogenated ethanes and ethylenes in the modified vapor phase HPC/DNA repair test, vinyl chloride was weakly active at the concentrations tested, and in the absence of cytotoxicity. Reduction in exposure time did not reduce the degree of cell killing but resulted in more extensive cell killing effects of some of these compounds, suggesting that maximum intracellular concentrations may be achieved very rapidly. Neither 1,1,1-trichloroethane or 1,1,2-trichloroethylene with, without or with low stabilizers, induced repair synthesis. However, increased grain counts were obtained with both non-stabilized and stabilized tetrachloroethylene at concentrations exhibiting cytotoxic activity. As shown in the bacterial studies, extraneous materials in the samples of tetrachloroethylene may be responsible for their UDS activity.

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In the conventional liquid phase HPC/DNA repair test, the test compounds produced no repair synthesis, although the positive control, 2-acetylaminofluorene, was highly active. Nevertheless, the compounds were cytotoxic as with vapor phase exposure. The absence of positive results with tetrachloroethylene, as opposed to those obtained with vapor phase exposure, may indicate that the impurities postulated to be responsible for this effect are either poorly soluble or highly volatile.

In summary, based on the present results, we find no evidence that 1,1,1-trichloroethane, 1,1,2-trichloroethylene and tetrachloroethylene per se are genotoxic.

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TABLE I

LIST OF TEST COMPOUNDS

Chemical	Container	Assay
Vinyl Chloride (VC)	Stainless Steel Cylinder	99.99%
1,1,2-Trichloroethylene (TCE)		
Stabilized (Trichlor 136)	Amber Glass Bottle	--
Non-stabilized (Trichlor 119)	"	99.90%
1,1,1-Trichloroethane		
Stabilized (Triethane 324A)	"	--
Low-stabilized (Triethane 321)	"	99.80%
Tetrachloroethylene (Perchloroethylene)		
Stabilized (Perchlor 230)	"	--
Non-stabilized (Perchlor 200)	"	99.93%
High purity	"	99.99+%

More detailed assay information on these materials will be maintained only by PPG for proprietary reasons.

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TABLE II

GAS CHROMATOGRAPHY ANALYSIS OF VINYL CHLORIDE CONCENTRATION IN THE CHAMBER

Calculated Concentration[a]	Actual Concentration[b]	
	0 Time	17 hrs.
10%	17.6%	15.9%
5%	7.24%	6.03%
1%	0.99%	0.99%
control (blank)	Not Detected	

[a] Vapor equivalent of liquid was calculated according to the equation described in Materials and Methods.

[b] Gas samples were obtained at time 0, the immediately after the dissipation of gas phase vinyl chloride inside of the chamber and 17 hrs after the incubation at 37°C.

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TABLE III

GAS CHROMATOGRAPHY ANALYSIS OF 1,1,1-TRICHLOROETHANE, AND 1,1,2-TRICHLOROETHYLENE

Compound	Actual Concentration[a]	
	0 Time	17 hours
1,1,1-Trichloroethane low-stabilized	5.0%	4.2 %
1,1,2-Trichloroethylene non-stabilized	5.0%	4.6 %

[a] Vapor equivalent of liquid was calculated according to the equation described in Materials and Methods. The concentration of gas in air in chamber was analysed by gas chromatography at 0 time (immediately after the dissipation of gas inside the chamber), and at 17 hours.

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TABLE IV-a

SALMONELLA/MICROSOME MUTAGENESIS ASSAYS ON HALOGENATED ETHANES AND ETHYLENES BY MODIFIED EXPOSURE METHOD[a]

Compound	Conc (%)	No. of induced revertants per plate[b] TA 100 No S-9		TA 100 With S-9		TA 1535 No S-9		TA 1535 With S-9	
Vinyl Chloride	10.0	192	(100%)[c]	494	(100%)	167	(100%)	271	(100%)
	7.5	190	(100%)	686	(74%)	201	(100%)	955	(78%)
	5.0	209	(75%)	413	(86%)	77	(84%)	217	(98%)
	2.5	69	(80%)	208	(85%)	113	(100%)	170	(100%)
	1.0	20	(100%)	120	(100%)	79	(77%)	118	(90%)
	0.1	34	(100%)	112	(100%)	54	(100%)	37	(90%)
1,1,1 Trichloroethane low-stabilized	10.0	370	(1.4%)	423	(0.4%)	73	(4.5%)	98	(1.3%)
	7.5	28	(1.8%)	93	(2.2%)	5	(4.1%)	2	(1.7%)
	5.0	0	(79%)	2	(89%)	12	(69%)	0	(70%)
	2.5	0	(86%)	14	(100%)	6	(90%)	1	(94%)
stabilized	10.0	92	(2.5%)	113	(1.5%)	25	(1.3%)	20	(2.2%)
	7.5	314	(1.2%)	416	(0.9%)	247	(7.4%)	234	(4.8%)
	5.0	302	(71%)	461	(74%)	338	(89%)	366	(68%)
	2.5	185	(93%)	315	(87%)	248	(100%)	198	(95%)
	1.0	69	(100%)	146	(71%)	12	(81%)	0	(97%)
1,1,2 Trichloroethylene non-stabilized	10.0	No Growth							
	7.5	No Growth							
	5.0	0	(2.6%)	0	(2.7%)	0	(4.4%)	0	(2.6%)
	2.5	0	(21%)	0	(14%)	7	(41%)	0	(21%)
	1.0	7	(95%)	57	(95%)	31	(91%)	17	(95%)
stabilized	10.0	No Growth							
	7.5	No Growth							
	5.0	722	(2.8%)	769	(3.0%)	939	(4.7%)	904	(3.6%)
	2.5	23	(18%)	36	(17%)	116	(33%)	109	(41%)
	1.0	17	(41%)	42	(46%)	43	(88%)	25	(88%)

[a] The assays were performed according to the method described by Ames (1) with slight modifications as described in Materials and Methods. Tester stains with S-9 mix or with buffer solution were poured onto basal agar medium and incubated at 37°C for 2 1/2 hrs. to allow initiation of cell growth, then exposed to gas phase of each compound in tightly sealed chamber for 18 hrs.

[b] Average of 2 experiments each consisting of 3 replicate plates. Figures represent total revertants minus spontaneous revertants.

[c] Surviving fraction of cells as determined by simultaneous cytotoxicity test. "No growth" indicate no surviving cells on cytotoxicity test plate.

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TABLE IV-b

SALMONELLA/MICROSOME MUTAGENESIS ASSAYS ON HALOGENATED ETHANES AND ETHYLENES BY MODIFIED EXPOSURE METHOD[a]

Compound	Conc (%)	No. of induced revertant per plate[b] TA 100 No S-9		TA 100 With S-9		TA 1535 No S-9		TA 1535 With S-9	
Tetrachloroethylene non-stabilized	10.0	No Growth							
	7.5	No Growth							
	5.0	No Growth							
	2.5	243	(1.9%)	269	(1.1%)	135	(1.5%)	140	(1.1%)
	1.0	11	(26%)	34	(31%)	16	(24%)	9	(26%)
	0.1	0	(71%)	0	(82%)	10	(77%)	3	(83%)
stabilized	10.0	No Growth							
	7.5	No Growth							
	5.0	No Growth							
	2.5	315	(2.3%)	356	(1.7%)	199	(1.8%)	240	(2.2%)
	1.0	0	(20%)	0	(33%)	8	(41%)	0	(29%)
	0.1	0	(71%)	0	(80%)	7	(90%)	0	(84%)
Tetrachloroethylene High Purity	2.5	No Growth							
	1.0	0	(0.6%)	0	(4.0%)	11	(14%)	24	(22%)
	0.1	0	(4.0%)	19	(10%)	19	(39%)	24	(55%)
Spontantous Revertants		177		157		20		24	

[a]The assays were performed according to the method described by Ames (1) with slight modifications as described in Materials and Methods. Tester stains mixed with S-9 mix or with buffer solution were poured onto basal agar medium and incubated at 37°C for 2 1/2 hrs. to allow initiation of cell growth, then exposed to gas phase of each compound in tightly sealed chamber for 18 hours.

[b]Average of 2 experiments, each consisting of 3 replicate plates. Figures represent total revertants minus spontaneous revertants.

[c]Surviving fraction of cells as determined by simultaneous cytotoxicity test. "No growth" indicates no surviving cells on cytotoxicity test plate.

R&S 024337

TABLE V

SALMONELLA/MICROSOME MUTAGENESIS ASSAYS ON HALOGENATED ETHANES AND ETHYLENES BY MODIFIED EXPOSURE METHOD[a]

Compound	Conc.(%)	No. of induced revertants per plate[b]							
		TA 98		TA 1538		TA 1537	
		No S-9	With S-9	No S-9	With S-9	No S-9	With S-9
Vinylchloride	7.5	10 (100%)[c]	17 (100%)	0 (100%)	10 (100%)	4 (80%)	1 (100%)
1,1,1-Trichloroethane low-stabilized	10.0	6 (17%)	12 (68%)	19 (17%)	10 (18%)	0 (10%)	0 (7.8%)
stabilized	5.0	3 (84%)	9 (94%)	0 (100%)	0 (100%)	0 (91%)	0 (76%)
1,1,2-Trichloroethylene non-stabilized	2.5	0 (30%)	0 (23%)	0 (48%)	1 (46%)	0 (50%)	0 (52%)
stabilized	2.5	2 (34%)	2 (22%)	0 (23%)	0 (23%)	0 (11%)	1 (11%)
Tetrachloroethylene non-stabilized	1.0	10 (47%)	6 (49%)	3 (39%)	3 (39%)	8 (39%)	4 (45%)
stabilized	1.0	9 (43%)	12 (35%)	0 (40%)	4 (39%)	1 (36%)	1 (40%)
Spontaneous Revertants		47	47	20	19	8	8

[a] The assays were performed according to the method described by Ames (1) with slight modifications as described in Materials and Methods. Tester stains with S-9 mix or with buffer solution were ponted onto basal agar medium and incubated at 37°C for 2 1/2 hrs. to allow initiation of cell growth, then exposed to gas phase of each compound in tightly sealed chamber for 18 hrs.

[b] Average of 2 experiments each consisting of 3 replicate plates. Figures represent total revertants minus spontaneous revertants.

[c] Surviving fraction of cells as determined by simultaneous cytotoxicity test. "No growth" indicate no surviving cells on cytotoxicity test plate.

R&S 024338

TABLE VI

HPC/DNA REPAIR ASSAYS ON HALOGENATED ETHANES AND ETHYLENES BY MODIFIED EXPOSURE METHOD[a]

Compound	Conc.(%)[b]	Exposure Time 3 hrs. Autoradiograph Grains/Nucleus	3 hrs. Cytotoxicity	18 hrs. Autoradiograph Grains/Nucleus	18 hrs. Cytotoxicity
Vinyl Chloride	5.0	Not Done		11.3±1.9 [c]	0
	2.5	Not Done		4.9±3.5	0
1,1,1 Trichloroethane low-stabilized	2.5	Not Countable	4+	2.3±2.0	1+
	1.0	0.6±1.7	4+	4.6±3.8	0
	0.1	0.9±2.2	3+	3.1±0.9	0
stabilized	5.0	1.3±5.8	4+	Not Done	
	2.5	1.1±2.8	3+	Not Countable	4+
	1.0	0	2+	.84±.7	1+
	0.1	0	0	.76±.4	1+
1,1,2 Trichloroethylene non-stabilized	2.5	0	2+	.20±.2	4+
	1.0	0	2+	3.2±2.5	4+
	0.1	.92±1.8	2+	2.5±.9	4+
stabilized	2.5	0.2±0.4	2+	Not Countable	4+
	1.0	1.0±1.8	2+	1.2±3.3	0
	0.1	1.0±2.2	2+	2.8±1.3	0
Tetrachloroethylene non-stabilized	2.5	Not Countable	4+	Not Countable	4+
	1.0	Not Countable	4+	Not Countable	4+
	0.1	10.8±6.1	3+	2.5±.9	1+
stabilized	2.5	Not Countable	4+	Not Countable	4+
	1.0	Not Countable	4+	Not Countable	4+
	0.1	6.2±4.9	2+	15.9±1.6	1+

[a] Cells attached on glass coverslips in reduced volume of culture medium were exposed to gas phase of each compound in tightly sealed chamber for designated period of time. Modification of the method developed by Williams (3).

[b] Each compound was introduced into the chamber in liquid phase at the volume that produce desired concentration in gas phase according to the equation described in Materials and Methods.

[c] Average of 2-3 coverslips of each, 20 nucleus were counted.

R&S 024339

TABLE VII-a

HPC/DNA REPAIR ASSAYS ON HALOGENATED ETHANES AND ETHYLENES BY CONVENTIONAL METHOD[a]

Compound	Conc.(%)	Exposure Time 3 hrs. Autoradiograph Grains/Nucleus	3 hrs. Cytotoxicity	18 hrs. Autoradiograph Grains/Nucleus	18 hrs. Cytotoxicity
1,1,1 Trichloroethane					
low-stabilized	1.0	Not Countable	4+	Not Countable	4+
	0.1	Not Countable	4+	Not Countable	4+
	0.01	Not Countable	2+	.5 ±.4	1+
	0.001	0	2+	.9 ±.2	0
	0.0001	0	0	0	0
stabilized	1.0	Not Countable	4+	Not Countable	4+
	0.1	0.1 ±0.2	2+	Not Countable	4+
	0.01	0	2+	.3 ±.5	4+
	0.001	0	0	.7 ±.7	0
1,1,2 Trichloroethylene					
non-stabilized	1.0	Not Countable	4+		
	0.1	Not Countable	4+	Not Done	
	0.01	0	4+	Not Countable	4+
	0.001	0	2+	.2 ±.9	1+
stabilized	1.0	Not Countable	4+	Not Countable	4+
	0.1	Not Countable	4+	Not Countable	4+
	0.01	0	2+	.6 ± .8	1+
	0.001	0	0	.7 ±1.0	0

[a] The assays were performed according to the method described by Williams (3) with slight modifications as described in Materials and Methods. Vinyl chloride was not tested by this method.
[b] Each compound was introduced into the petri dish in liquid phase to have desired final concentration.
[c] Positive control.
[d] Negative control.

R&S 02434O

TABLE VII-b

HPC/DNA REPAIR ASSAYS ON HALOGENATED ETHANES AND ETHYLENES BY CONVENTIONAL METHOD[a]

Compound	Conc.(%)	Exposure Time: 3 hrs. Autoradiograph Grains/Nucleus	3 hrs. Cytotoxicity	18 hrs. Autoradiograph Grains/Nucleus	18 hrs. Cytotoxicity
Tetrachloroethylene					
non-stabilized	1.0	Not Countable	4+	Not Done	
	0.1	Not Countable	4+	Not Countable	4+
	0.01	Not Countable	4+	Not Countable	4+
	0.001	0	3+	.2 ±.1	1+
	0.0001	1.0±2.23	3+	.4 ±.2	1+
stabilized	1.0	Not Countable	4+	Not Done	
	0.1	Not Countable	4+	Not Done	
	0.01	Not Countable	4+	1.8±1.0	4+
	0.001	0	2+	.1± .1	1+
2 Acetylaminofluorene[c]	10^{-5}M	169.0±74.9	0	170.2±20.6	0
Fluorene[d]	10^{-5}M	Not Done		1.6± .6	0
Pyrene	10^{-5}M	0	0	Not Done	
Cell Control	0	0	0	2.75±2.76	0

[a] The assays were performed according to the method described by Williams (3) with slight modifications as described in Materials and Methods. Vinyl chloride was not tested by this method.
[b] Each compound were introduced into the petri dish in liquid phase to have desired final concentration.
[c] Positive control.
[d] Negative control.

R&S 02434

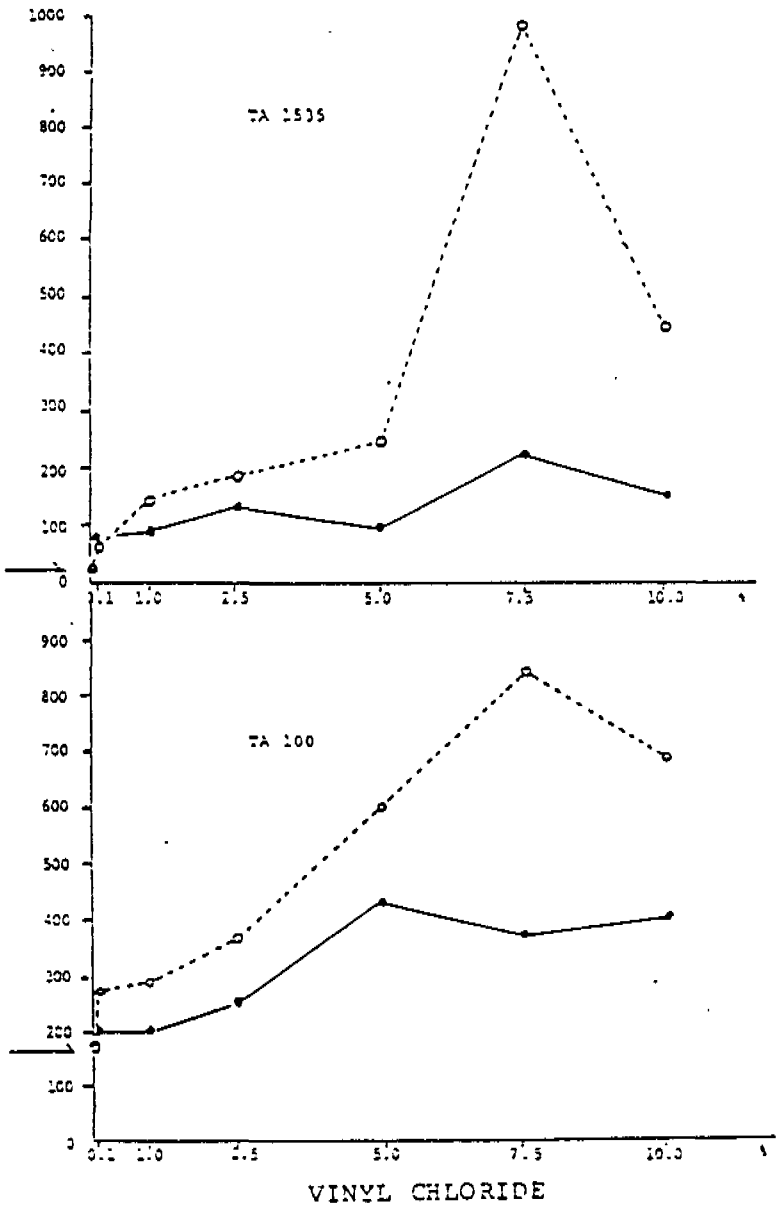
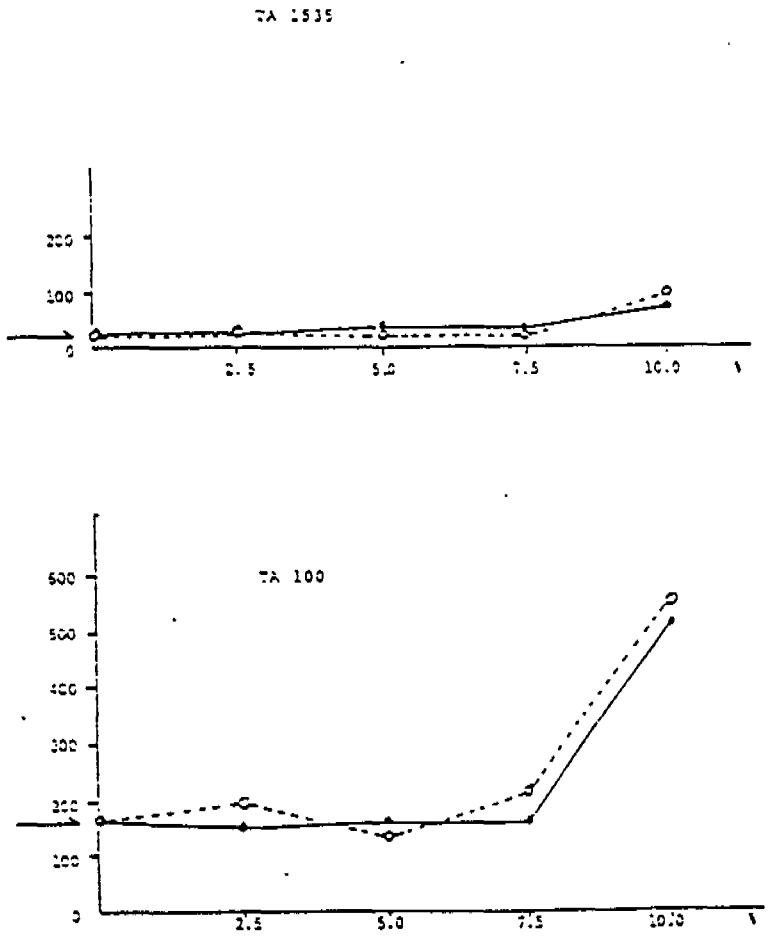


Fig.1. Dose-response curves for vinyl chloride with Salmonella typhimurium tester strains with (--o--) and without (—•—) activation system containing 150µl of Aroclor induced rat S-9. Each point represents the geometric mean of triplicate plates from one experiment. Spontaneous revertants are indicated by arrows.

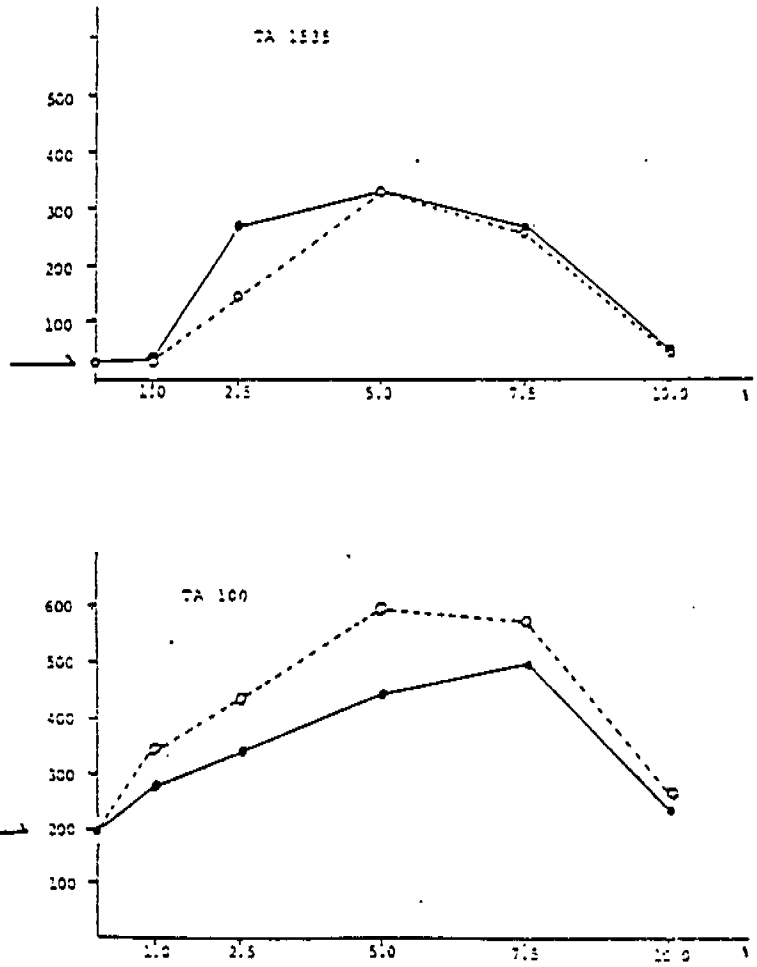
R&S 024342



1,1,1-TRICHLOROETHANE, Low-stabilized

Fig.2. Dose-response curves for 1,1,1-trichloroethane with low concentrations of stabilizer with Salmonella typhimurium tester strains with (--o--) and without (—•—) activation system containing 150ul of Aroclor induced rat S-9. Each point represents the geometric mean of triplicate plates from one experiment. Spontaneous revertants are indicated by arrows.

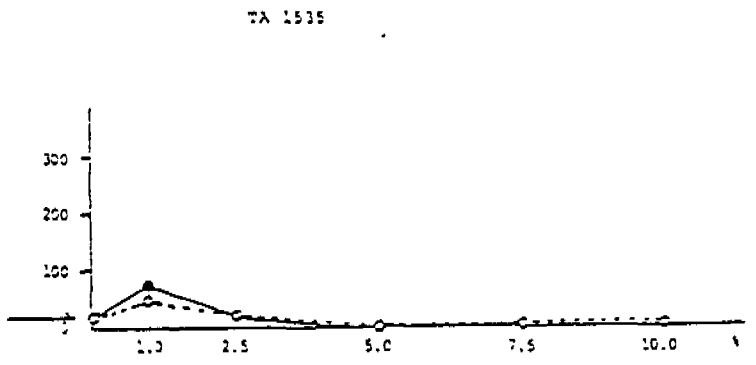
R&S 024343

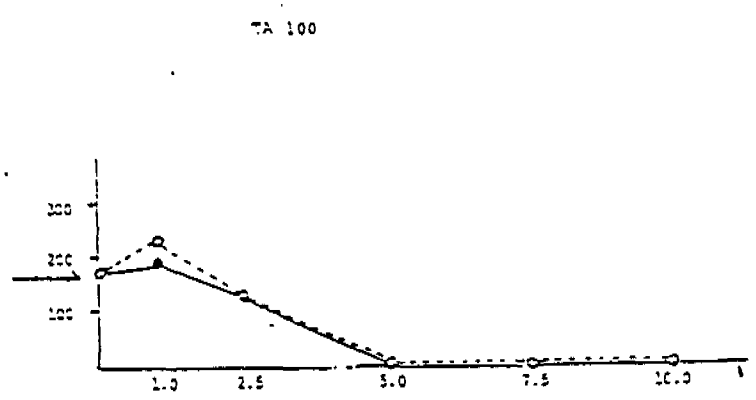


1,1,1-TRICHLOROETHANE, Stabilized

Fig.3. Dose-response curves for 1,1,1-trichloroethane with stabilizer with Salmonella typhimurium tester strains with (--o--) and without (—•—) activation system containing 150µl of Aroclor induced rat S-9. Each point represents the geometric mean of triplicate plates from one experiment. Spontaneous revertants are indicated by arrows.

R&S 024344

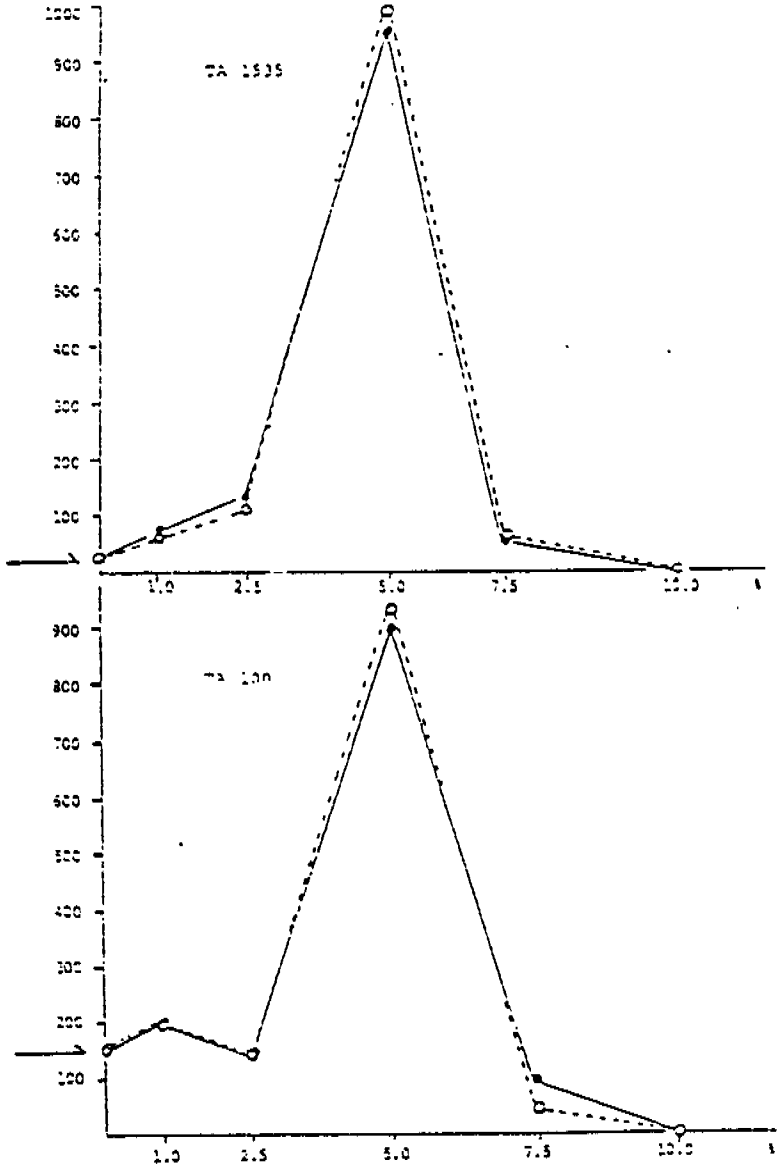




1,1,2-TRICHLOROETHYLENE, Non-stabilized

Fig.4. Dose-response curves for 1,1,1-trichloroethylene without stabilizer with Salmonella typhimurium tester strains with (--o--) and without (—•—) activation system containing 150ul of Aroclor induced rat S-9. Each point represents the geometric mean of triplicate plates from one experiment. Spontaneous revertants are indicated by arrows.

R&S 024345

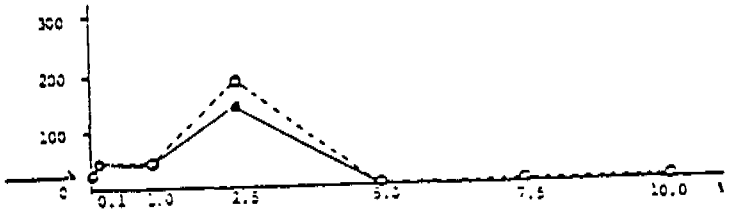


1,1,2-TRICHLOROETHYLENE, Stabilized

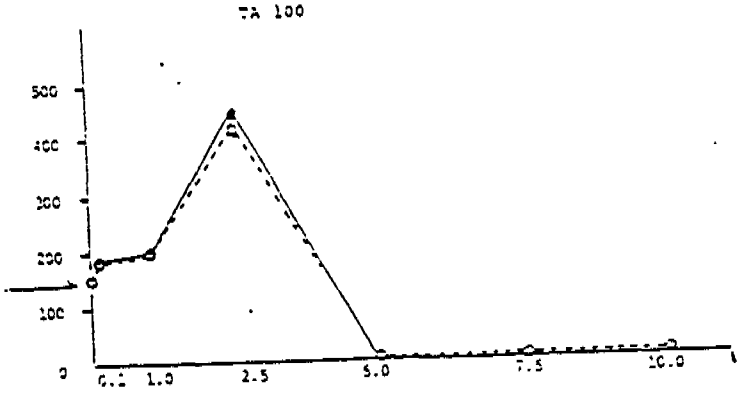
Fig.5. Dose-response curves for 1,1,2-trichloroethylene with stabilizer with Salmonella typhimurium tester strains with (--o--) and without —●—) activation system containing 150ul of Aroclor induced rat S-9. Each point represents the geometric mean of triplicate plates from one experiment. Spontaneous revertants are indicated by arrows.

R&S 024347

TA 1535



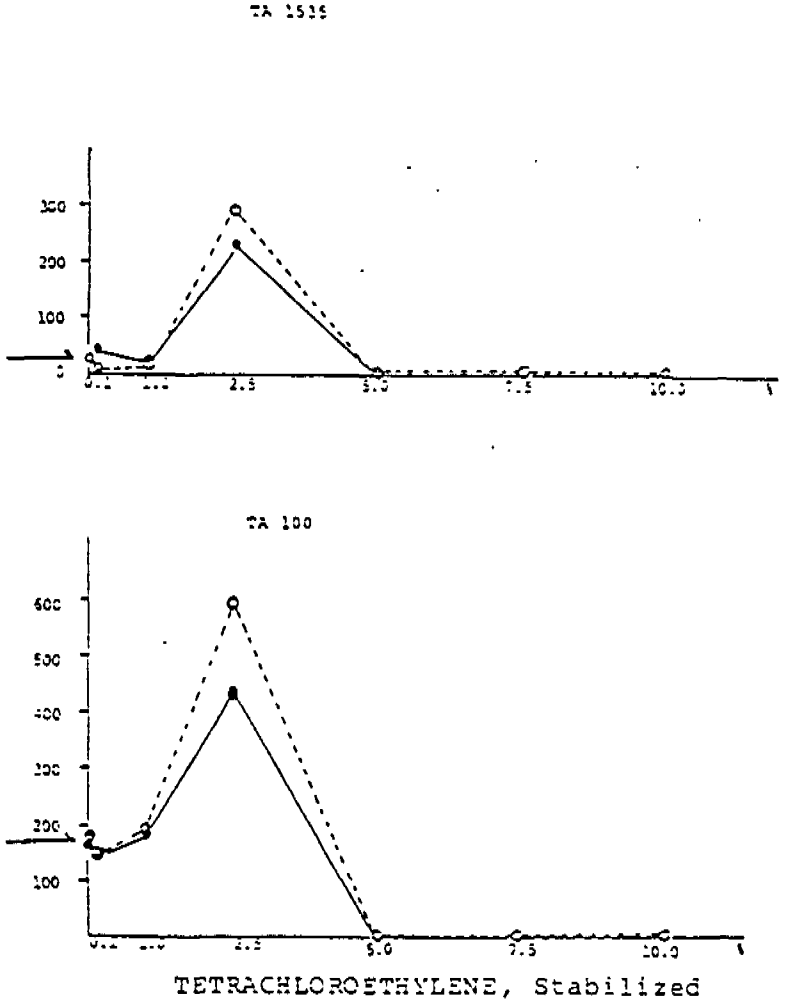
TA 100



TETRACHLOROETHYLENE, Non-stabilized

Fig.6. Dose-response curves for tetrachloroethylene without stabilizer with Salmonella typhimurium tester strains with (--o--) and without (—•—) activation system containing 150µl of Aroclor induced rat S-9. Each point represents the geometric mean of triplicate plates from one experiment. Spontaneous revertants are indicated by arrows.

R&S 024348



TETRACHLOROETHYLENE, Stabilized

Fig.7. Dose-response curves for tetrachloroethylene with stabilizer with Salmonella typhimurium tester strains with (--o--) and without (—•—) activation system containing 150 µl of Aroclor induced rat S-9. Each point represents the geometric mean of triplicate plates from one experiment. Spontaneous revertants are indicated by arrows.

APPENDIX I-a: SUMMARY OF FIRST SET OF EXPERIMENTS

SALMONELLA/MICROSOME MUTAGENESIS ASSAYS ON HALOGENATED ETHANES AND ETHYLENES[a]

Compound	Conc.(%)	Number of Total Revertants per Plate			
		TA-100		TA-1535	
		without S-9	with S-9	without S-9	with S-9
Vinyl Chloride	10.0	297, 330, 369[b]	738, 678, 762	174, 159, 138	258, 300, 369
	7.5	375, 354, 372	813, 837, 879	225, 210, 228	894,1014,1029
	5.0	408, 468, 414	684, 690, 435	93, 105, 93	225, 315, 183
	2.5	222, 267, 249	354, 369, 372	120, 156, 123	195, 192, 195
	1.0	213, 171, 207	306, 264, 285	90, 114, 63	126, 138, 162
	0.1	210, 237, 186	279, 252, 276	75, 66, 81	63, 60, 60
1,1,1-Trichloroethane low-stabilized	10.0	534, 474, 531	489, 591, 555	78, 66, 72	111, 90, 96
	7.5	135, 117, 234	198, 288, 135	21, 33, 30	12, 21, 24
	5.0	180, 147, 156	105, 126, 168	30, 39, 39	24, 15, 33
	2.5	168, 135, 159	195, 162, 204	39, 27, 21	33, 15, 21
stabilized	10.0	294, 249, 159	258, 288, 240	42, 54, 45	33, 48, 60
	7.5	528, 432, 513	477, 600, 642	237, 324, 240	192, 264, 318
	5.0	429, 456, 447	555, 591, 612	318, 336, 342	313, 384, 264
	2.5	342, 321, 348	402, 495, 387	252, 300, 243	252, 246, 237
	1.0	288, 237, 306	354, 360, 297	27, 30, 33	30, 18, 27
1,1,2-Trichloroethylene non-stabilized	10.0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	7.5	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	5.0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	2.5	108, 150, 132	135, 96, 165	24, 36, 18	24, 30, 15
	1.0	246, 198, 129	267, 198, 231	72, 78, 75	60, 48, 48
stabilized	10.0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	7.5	102, 78, 90	75, 39, 18	90, 48, 30	120, 30, 36
	5.0	690,1020,1014	1023, 861, 912	1042, 837,1002	885,1101, 975
	2.5	153, 129, 144	138, 171, 117	138, 141, 117	90, 120, 114
	1.0	177, 180, 248	186, 165, 237	78, 75, 63	39, 61, 75
Spontaneous Revertants		183, 213, 174[c]	171, 150, 150	24, 24, 24	18, 18, 24

[a] The assays were performed according to the method described by Ames (1) with slight modifications as described in Materials and Methods. Tester stains with S-9 mix or with buffer solution were poured onto basal agar medium and incubated at 37°C for 2 1/2 hrs. to allow initiation of cell growth, then exposed to gas phase of each compound in tightly sealed chamber for 10 hrs.

[b] Total number of revertants for each triplicate.

[c] Spontaneous revertants for each triplicate from vinyl chloride 2.5% experiment.

RAS 02434g

APPENDIX I-b: SUMMARY OF FIRST SET OF EXPERIMENTS

SALMONELLA/MICROSOME MUTAGENESIS ASSAYS ON HALOGENATED ETHANES AND ETHYLENES[a]

Compound	Conc.(%)	Number of Total Revertants per Plate			
		TA-100		TA-1535	
		without S-9	with S-9	without S-9	with S-9
Tetrachloroethylene non-stabilized	7.5	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	5.0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	2.5	435, 403, 399[b]	405, 435, 423	147, 156, 126	162, 181, 181
	1.0	201, 147, 243	174, 225, 189	45, 51, 21	30, 33, 36
	0.1	159, 141, 147	180, 163, 171	24, 36, 48	27, 42, 28
stabilized	10.0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	7.5	Not Done	Not Done	Not Done	Not Done
	5.0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	2.5	468, 561, 534	606, 538, 531	294, 258, 264	321, 363, 345
	1.0	57, 36, 33	42, 30, 54	18, 30, 24	18, 12, 18
	0.1	129, 153, 144	144, 147, 156	24, 33, 45	12, 12, 18
Tetrachloroethylene high purity	2.5	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	1.0	99, 84, 87	81, 75, 96	24, 36, 21	48, 36, 54
	0.1	123, 138, 156	153, 144, 186	27, 42, 33	30, 42, 48
Spontaneous Revertants		182, 213, 174	171, 150, 150	24, 24, 24	18, 18, 24

[a] The assays were performed according to the method described by Ames (1) with slight modifications as described in Materials and Methods. Tester stains with S-9 mix or with buffer solution were poured onto basal agar medium and incubated at 37°C for 2 1/2 hrs. to allow initiation of cell growth, then exposed to gas phase of each compound in tightly sealed chamber for 18 hrs.

[b] Total number of revertants for each triplicate.

[c] Spontaneous revertants for each triplicate from vinyl chloride 2.5% experiment.

R&S 024350

APPENDIX II-a: SUMMARY OF SECOND SET OF EXPERIMENTS

SALMONELLA/MICROSOME MUTAGENESIS ASSAYS ON HALOGENATED ETHANES AND ETHYLENES[a]

Compound	Conc.(%)	Number of Total Revertants per Plate			
		TA-100		TA-1535	
		without S-9	with S-9	without S-9	with S-9
Vinyl chloride	20.0	453, 429, 420[b]	558, 525, 585	207, 240, 204	309, 258, 278
	10.0	438, 384, 396	618, 633, 594	171, 183, 198	276, 276, 321
	5.0	312, 360, 351	573, 585, 450	81, 102, 99	210, 181, 198
1,1,1Trichloroethane low-stabilized	10.0	603, 561, 579	621, 615, 606	102, 123, 114	177, 120, 135
	7.5	231, 303, 210	333, 294, 252	27, 15, 24	30, 30, 39
	5.0	151, 186, 219	213, 174, 168	33, 27, 21	30, 21, 15
	2.5	102, 135, 207	114, 153, 195	12, 30, 24	24, 24, 33
stabilized	10.0	279, 312, 321	276, 288, 267	48, 54, 27	42, 48, 30
	7.5	528, 432, 513	477, 600, 642	237, 324, 240	192, 264, 318
	5.0	468, 489, 582	657, 627, 663	399, 363, 387	471, 465, 441
	2.5	372, 351, 435	471, 534, 543	252, 282, 276	162, 225, 207
	1.0	165, 252, 228	282, 261, 264	21, 36, 42	27, 12, 30
1,1,2-Trichloroethylene non-stabilized	7.5	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	5.0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	2.5	165, 183, 174	138, 174, 171	30, 33, 21	18, 30, 24
	1.0	180, 177, 174	186, 180, 213	24, 18, 36	21, 27, 42
stabilized	7.5	93, 78, 99	72, 90, 26	63, 51, 48	45, 36, 60
	5.0	894, 861, 915	1026, 825, 906	933, 963, 978	636, 1041, 927
	2.5	258, 234, 282	174, 282, 273	177, 132, 111	147, 171, 153
	1.0	210, 147, 204	189, 210, 207	39, 54, 66	27, 36, 54
Spontaneous Revertants		183, 144[c]	150, 120	15, 15	30, 24

[a] The assays were performed according to the method described by Ames (1) with slight modifications as described in Materials and Methods. Tester stains with S-9 mix or with buffer solution were poured ontobasal agar medium and incubated at 37°C for 2 1/2 hrs. to allow initiation of cell growth, then exposed gas phase of each compound in tightly sealed chamber for 18 hrs.

[b] Total number of revertants for each triplicate.

[c] Spontaneous revertants for each duplicate from vinyl chloride 5% experiment.

R&S 024351

APPENDIX II-b: SUMMARY OF SECOND SET OF EXPERIMENTS

SALMONELLA/MICROSOME MUTAGENESIS ASSAYS ON HALOGENATED ETHANES AND ETHYLENES[a]

Compound	Conc.(%)	Number of Total Revertants per Plate			
		TA-100		TA-1535	
		without S-9	with S-9	without S-9	with S-9
Tetrachloroethylene non-stabilized	2.5	384, 396, 423[b]	417, 468, 408	183, 144, 174	138, 171, 144
	1.0	201, 159, 177	177, 195, 186	36, 39, 24	24, 30, 42
	0.1	144, 150, 165	144, 153, 117	24, 21, 24	18, 21, 24
stabilized	2.5	444, 477, 468	411, 468, 525	165, 144, 186	177, 189, 186
	1.0	225, 138, 183	183, 189, 195	36, 36, 21	30, 42, 24
	0.1	114, 138, 171	141, 156, 180	21, 18, 18	24, 24, 24
Tetrachloroethylene high purity	2.5	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	1.0	69, 105, 54	102, 90, 75	42, 21, 42	87, 81, --
	0.1	141, 138, 150	165, 213, 192	54, 42, 36	48, 54, 66
Spontaneous Revertants		183, 144[c]	150, 120	15, 15	30, 24

[a] The assays were performed according to the method described by Ames (1) with slight modifications a described in Materials and Methods. Tester stains with S-9 mix or with buffer solution were poured onto basal agar medium and incubated at 37°C for 2 1/2 hrs. to allow initiation of cell growth, the exposed to gas phase of each compound in tightly sealed chamber for 18 hrs.

[b] Total number of revertants for each triplicate.

[c] Spontaneous revertants for each duplicate from vinyl chloride 5% experiment.

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APPENDIX III: SUMMARY OF FRAME SHIFT MUTATION TESTS

SALMONELLA/MICROSOME MUTAGENESIS ASSAYS ON HALOGENATED ETHANES AN ETHYLENES[a]

Compound	Conc.(%)	Number of Total Revertants per Plate					
		TA-100		TA-1535		TA-1537	
		without S-9	with S-9	without S-9	with S-9	without S-9	with S-9
Vinyl chloride	7.5	57, 81, 54[b]	48, 72, 51	21, 15, 15	27, 36, 27	3, 9, 12	6, 9, 9
1,1,1-Trichloroethane low-stabilized	10.0	60, 45, 48	45, 42, 54	51, 30, 36	36, 27, 18	9, 3, 6	6, 12, 0
stabilized	5.0	27, 30, 33	22, 42, 42	18, 36, 21	15, 21, 15	15, 12, 3	6, 12, 6
1,1,2-Trichloroethylene non-stabilized	2.5	30, 57, 36	42, 45, 33	15, 24, 27	27, 21, 18	3, 3, 9	3, 0, 6
stabilized	2.5	42, 54, 36	45, 45, 42	24, 27, 24	15, 12, 21	9, 12, 15	6, 12, 12
Tetrachloroethylene non-stabilized	1.0	45, 30, 42	42, 36, 36	33, 15, 24	15, 18, 30	15, 18, 15	6, 18, 12
stabilized	1.0	57, 42, 48	45, 48, 51	24, 12, 18	30, 15, 27	12, 15, 6	9, 9, 9
Spontaneous Revertants		33, 60, 48[c]	54, 42, 45	21, 15, 21	15, 15, 30	12, 6, 9	12, 12, 12

[a] The assays were performed according to the method described by Ames (1) with slight modifications as described in Materials and Methods. Tester stains with S-9 mix or with buffer solution were poured onto basal agar medium and incubated at 37°C for 2 1/2 hrs. to allow initiation of cell growth, then exposed to gas phase of each compound in tightly sealed chamber for 18 hrs.

[b] Total number of revertants for each triplicate.

[c] Spontaneous revertants for each triplicate from vinyl chloride experiment.

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APPENDIX III: SUMMARY OF FRAME SHIFT MUTATION TESTS

SALMONELLA/MICROSOME MUTAGENESIS ASSAYS ON HALOGENATED ETHANES AND ETHYLENES[a]

Compound	Conc.(%)	Number of Total Revertants per Plate					
		TA-98		TA-1538		TA-1537	
		without S-9	with S-9	without S-9	with S-9	without S-9	with S-9
Vinyl chloride	7.5	57,81,54[b](180%)[c]	48,72,51(120%)	21,15,15(120%)	27,36,27(103%)	3, 9,12 (80%)	6, 9, 9 (109%)
1,1,1-Trichloroethane							
low-stabilized	10.0	60,45,48 (17%)	45,42,54 (68%)	51,30,36 (17%)	36,27,18 (18%)	9, 3, 6 (10%)	6,12, 0 (7.8%)
stabilized	5.0	27,30,33 (84%)	22,42,42 (94%)	18,36,21(108%)	15,21,15(100%)	15,12, 3 (91%)	6,12, 6 (76%)
1,1,2-Trichloroethylene							
non-stabilized	2.5	30,57,36 (50%)	42,45,33 (52%)	15,24,27 (48%)	27,21,18 (46%)	3, 3, 9 (30%)	3, 0, 6 (23%)
stabilized	2.5	42,54,36 (34%)	45,45,42 (22%)	21,27,24 (23%)	15,12,21 (11%)	9,12,15 (11%)	6,12,12 (11%)
Perchloroethylene							
non-stabilized	1.0	45,30,42 (41%)	42,36,36 (49%)	33,15,24 (39%)	15,18,30 (39%)	15,18,15 (39%)	6,18,12 (45%)
stabilized	1.0	57,42,48 (43%)	45,48,51 (35%)	24,12,18 (40%)	30,15,27 (39%)	12,15, 6 (36%)	9, 9, 9 (40%)
Spontaneous Revertants		33,60,48[d]	54,42,45	21,15,21	15,15,30	12, 6, 9	12,12,12

[a] The assays were performed according to the method described by Ames (1) with slight modifications as described in Materials and Methods. Tester stains with S-9 mix or with buffer solution were poured onto basal agar medium and incubated at 37°C for 2 1/2 hrs. to allow initiation of cell growth, then exposed to gas phase of each compound in tightly sealed chamber for 18 hrs.

[b] Total number of revertants for each triplicate.

[c] The surviving fraction of cells at that concentration.

[d] Spontaneous revertants for each triplicate from vinyl chloride experiment.

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