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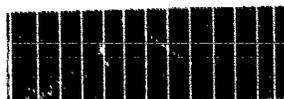
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Subject: Trichloroethylene Substantial Risk Report

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This letter and attachments summarize information which we believe to be reportable under Section 8(e) of the Toxic Substances Control Act (TSCA).

As part of an effort to study the toxicology and biochemical effects of trichloroethylene, a series of in-vivo and in-vitro tests were performed at ICI's Central Toxicology Laboratory (CTL) in the U.K. A copy of a report on the results of these studies was recently made available to ICI Americas Inc. by our parent company, ICI Plc. The studies conducted at CTL have shown a specific lung lesion following inhalation of trichloroethylene. The lesion, which is characterized by vacuolation of the Clara cell, has been observed in female CD-1 mice exposed for a single six hour period to trichloroethylene concentrations ranging from 20-2,000 ppm. The effects were dose - related, with very few Clara cells being affected following exposure to 20 ppm. Mice exposed at concentrations of 100 ppm trichloroethylene and above showed a reduction in the cytochrome P-450 activity at the Clara cell, while glutathione-S-transferase activities were generally unaffected. The effect was specific to the Clara cell, no damage being apparent to other cell types.

Metabolism studies using isolated mouse Clara cells in culture showed the same metabolic pathway for trichloroethylene as found in the liver, i.e., from trichloroethylene to chloral, to trichloroethanol (and its glucuronide) and on to trichloroacetic acid. However, the formation of trichloroethanol, and in particular its glucuronide, from chloral was much reduced leading to an accumulation of chloral in the Clara ce

When tested in-vivo, it was shown that chloral was the only me...lite of trichloroethylene to generate the mouse lung Clara cell lesion,

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A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

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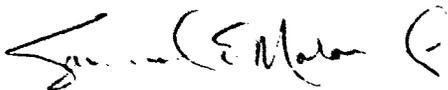
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trichloroethanol and trichloroacetic acid having no effect. The parent trichloroethylene is not expected to generate the lesion directly and indeed showed no effect in the rat exposed to up to 1000 ppm. Chloral is the product of P-450 enzyme metabolism of trichloroethylene, and mouse lung Clara cells possess a high level of P-450 activity. The few Clara cells found in human lung tissue show no smooth endoplasmic reticulum and are thus presumed not to possess significant P-450 activity, i.e., that required to generate chloral from trichloroethylene. Thus, the lesion observed in the mouse lung Clara cell resulting from production and accumulation of chloral would not be expected to occur in man. On this basis, the finding in mouse has no direct significance in human health hazard assessment.

No special actions involving notification to workers or efforts to reduce exposure to trichloroethylene are appropriate or planned by ICI on the basis of this report.

Respectfully submitted,



Samual E. Malovrh
Director, Environmental Affairs

JFJ/kt/.AK4

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Samual E. Malovrh
Director, Environmental Affairs

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A MECHANISM FOR THE DEVELOPMENT OF
CLARA CELL LESIONS IN THE MOUSE
LUNG AFTER EXPOSURE TO
TRICHLOROETHYLENE

by

T Green
J Odum
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Approved for Issue:


Product Manager

Date of Issue: 17 JUN 1991

CTL/R/1071 - 1

A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

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

T Green

(Study Director)

T. Green
.....

5.6.91.

A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

The following contributed to this report in the capacities indicated:

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A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
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A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

SUMMARY

Female CD-1 mice exposed to trichloroethylene (6hr/day) at concentrations from 20-2000ppm developed a highly specific lung lesion after a single exposure, characterised by vacuolation of the Clara cells, the number of cells affected increasing with increasing dose level. At the highest dose levels pyknosis of the Clara cells was apparent. After 5 days of repeated exposures the lesion had resolved but exposure of mice following a 2-day break resulted in the same lesion with increased severity. The changes in mouse lung Clara cells were accompanied by a marked loss of cytochrome P-450 activities. No morphological changes were seen in the lungs of rats exposed to either 500 or 1000ppm trichloroethylene. Isolated mouse lung Clara cells were shown to metabolize trichloroethylene to chloral, trichloroethanol and trichloroacetic acid. Chloral was the major metabolite. Trichloroethanol glucuronide was not detected. In comparative experiments using mouse hepatocytes the major metabolites were trichloroethanol and its glucuronide conjugate. The activity of UDP glucuronosyl transferases was compared in mouse lung Clara cells and hepatocytes using two phenolic substrates and trichloroethanol. Hepatocytes readily formed glucuronides from all three substrates whereas Clara cells were only active with the two phenolic substrates. The three major metabolites of trichloroethylene, chloral, trichloroethanol and trichloroacetic acid were each dosed to mice and of these metabolites, only chloral had an effect on mouse lung causing a lesion (Clara cell) identical to that seen with trichloroethylene. It is proposed that the failure of Clara cells to conjugate trichloroethanol leads to an accumulation of chloral which results in cytotoxicity. The known genotoxicity of chloral suggests that this lesion may be related to the development of lung tumours in mice exposed to trichloroethylene by inhalation.

1. INTRODUCTION

Trichloroethylene (TRI) is a volatile liquid which has been used extensively for many years, primarily as an industrial solvent. Over this period the toxicological evaluation of TRI has included a number of carcinogenicity bioassays (WHO, 1985). In the mouse, oral doses of TRI are known to induce hepatocellular carcinoma (NCI, 1976; NTP, 1983). Exposure by inhalation to (150 and 450ppm, 7 hours/day, 5 days/week for 104 weeks) resulted in increased incidences of lung adenomas and adenocarcinomas, but not liver tumours, in female CD-1 mice (Fukuda et al, 1983). In a study of shorter duration, B₆C₃F₁ and Swiss mice exposed to TRI (600ppm, 7 hours/day, 7 days/week for 78 weeks) developed both pulmonary adenomas and hepatocellular carcinomas (Maltoni et al 1986). In contrast to the findings in mice, the incidences of lung and liver tumours were not increased in rats exposed to TRI by either the oral or inhalation routes (NCI, 1976; Fukuda et al 1983, NTP, 1983; Maltoni et al 1986).

The acute effects of TRI on mouse lung have been investigated by Forkert et al (1985,1989). Administration of 2000mg/kg i.p. resulted in specific injury to the lung Clara cell with higher doses also affecting Type II alveolar cells. Lung microsomal cytochrome P-450 content and arylhydrocarbon hydroxylase activity were reduced. The Clara cell has been suggested to be the major site of cytochrome P-450 in the lung (Massey, 1989) and is the target of a number of chemicals which cause pulmonary lesions in various species. 4-Ipomeanol, naphthalene, carbon tetrachloride and 1,2-dichloroethylene, induce Clara cell lesions with other cell types largely unaffected. These compounds are all metabolized by cytochrome P-450 to reactive intermediates and the localization of P-450 in the Clara cell is believed to be responsible for the target cell specificity (Boyd,1977; Tong et al 1982; Boyd et al 1979; Forkert and Reynolds, 1982). TRI is also metabolized by cytochrome P-450 to chloral (hydrate) (Miller and Guengerich, 1982), which is in turn metabolized to trichloroethanol (TCE), trichloroethanol glucuronide and trichloroacetic acid (TCA) (Ikeda and Ohtsuji, 1972; Dekant et al 1984, Prout et al 1985). TCE-glucuronide

is the major urinary metabolite of TRI (approximately 90%) with TCA accounting for approximately 8% of the urinary metabolites (Green and Prout, 1985). Although the primary site of TRI metabolism is the liver, some metabolism and localization of TRI has been shown to occur in lung compartments (Bolt and Filser, 1977; Dalbey and Bingham, 1978; Bergman, 1983). This may be related to the pulmonary toxicity of TRI. Forkert et al (1989) have demonstrated covalent binding of TRI to lung macromolecules although at levels lower than in the liver. However, binding was assessed in the whole lung which may underestimate specific binding to Clara cells which only constitute a small proportion of total lung cells (Plopper 1983).

In the present study isolated mouse lung Clara cells and whole animal experiments have been used to investigate the basis of trichloroethylene induced lung lesions in mice and their possible relevance to the lung tumours seen in the 2 year bioassays is considered. The effects of trichloroethylene on the lungs of rats, a species where tumours are not seen, have also been investigated.

2. MATERIALS AND METHODS

2.1 Chemicals

1,1,2-Trichloroethylene (Aristar grade), anhydrous chloral and trichloroacetic acid (Analar grade) were obtained from BDH Chemicals Ltd (Poole, Dorset, UK). Trichloroethanol was obtained from Aldrich (Gillingham, Dorset, UK).

1,1,2-Trichloro[1,2-¹⁴C]ethylene, specific activity 20.4mCi/mole was obtained from Imperial Chemical Industries plc, Cambridge Research Biochemicals (Billingham, Cleveland, UK). The material had a chemical and radiochemical purity of greater than 98% as determined by radio-gas chromatography.

Biochemicals were obtained from Sigma Chemical Co. (Poole, Dorset, UK). Materials for hepatocyte isolation were obtained from Flow Laboratories (Irvine, Scotland, UK) and serum (foetal bovine serum and foetal calf serum) from Gibco (Grand Island NY, USA).

2.2 Animals

Female CD1 mice (20-25g) were supplied by Charles River (Margate, Kent, UK) and female Alpk:APfSD rats were obtained from ICI (Alderley Park, Cheshire, UK). The animals were housed in temperature controlled rooms fitted with a 12 hour lighting cycle. They received food (PCD diet, Special Diets Services Ltd, Witham, Essex, UK) and water ad libitum before and after but not during exposure.

2.3 Dosing

Trichloroethylene: Mice were given a single exposure to concentrations of 200, 450, 1000 or 2000ppm for 6 hours. In a separate study mice were exposed to 450ppm for 6h/day, 5 days/week for 2 weeks. Rats were given a single exposure to 500 or 1000ppm for 6 hours. Atmospheres were generated into glass dessicators by passing vapourized TRI into the input airstream. Air flow through the dessicators was approximately 7 litres/min. The atmospheric concentration of TRI was continuously monitored by infra-red analysis. Controls were exposed to air alone.

Chloral: Mice were given a single exposure to a concentrations of 100ppm for 6 hours. Atmospheres were generated and monitored as above.

Trichloroethanol: Mice were given a single exposure to concentrations of 100ppm for 6 hours or 500ppm for 2 hours. Atmospheres were generated as above and monitored by gas chromatography.

Trichloroacetic acid: Mice received a single dose of TCA 200 or 500mg/kg i.p. in 1% (w/v) Tween 80 (20ml/kg). The dosing solution was neutralized with sodium hydroxide before administration. Controls received 1% Tween 80 alone (20ml/kg i.p.).

In the single dose studies animals were killed 24 hours after the start of exposure, or dosing, and lungs removed. In the multiple exposure study animals were killed at the intervals shown in Figure 3. All animals were killed by overexposure to halothane followed by exsanguination, except for mice used for the preparation of hepatocytes which were killed by overexposure to diethyl ether.

2.4 Pathology

Lungs were removed, then inflated with and stored in 3% glutaraldehyde. They were dehydrated through an ascending ethanol series and embedded in paraffin wax. Sections (5µm) were cut and stained with haematoxylin and eosin for light microscopic examination.

2.5 Metabolism Studies

2.5.1 Preparation of Cells and Subcellular Fractions: Mouse lung Clara cells were prepared by the method of Oreffo et al (1990). Lungs from 6 mice were pooled for each preparation. The cell yield was approximately 1×10^6 cells/mouse. Preparations contained typically 85% Clara cells as judged by nitroblue tetrazolium staining (Devereux and Founts, 1980) of cytospin preparations. The final pellet of cells was resuspended in Hanks balanced salt solution (HBSS). Viability (95%) was determined by trypan blue exclusion. Cells prepared for determination of enzyme activities were resuspended in sucrose (250mM) EDTA (5.3mM) Tris-HCl (20mM) buffer pH 7.4 (SET), briefly sonicated and stored at -70°C until used.

Mouse hepatocytes were prepared by the method of Mitchell et al (1984). Cell yields were approximately 40×10^6 cells/mouse. The final pellet of cells was resuspended in a complete Liebowitz culture medium (CL15)

containing foetal calf serum (8.3%), tryptose phosphate broth (8.3%), penicillin (41.3IU/ml), streptomycin (82.6µg/ml), glutamine (241µg/ml), insulin (10^{-6} M), hydrocortisone (10^{-5} M) and Hepes (25mM). Viability, determined by trypan blue exclusion was approximately 85%.

Subcellular fractions of rat lung and mouse liver were prepared by homogenizing the whole tissue in SET with a teflon glass homogenizer, to give a 20% (w/v) homogenate. Washed microsomes and cytosol were then prepared as described previously (Green et al 1990) and stored at -70°C until used.

2.5.2 The Metabolism of TRI in Isolated Cells: Freshly isolated Clara cells or hepatocytes (1ml containing 10^6 cells) were incubated with ^{14}C -TRI (1mM, 20µCi in 1.4µl DMF) in sealed 10ml "reactiflasks" (Pierce, Chester, UK) at 37°C for up to 2 hours. Clara cells were incubated in HBSS and hepatocytes in CL15 medium. The reaction was stopped by addition of 4ml ice-cold diethyl ether and the flasks placed on ice. The flask contents were transferred to screw capped tubes and the flasks rinsed with water (1ml) which was added to the tubes. The tubes were shaken for 10 minutes and the phases separated. A further extraction was carried out with 4ml diethyl ether. Chloral and TCE were detected and quantified, with authentic standards, in the ether phases by gas chromatography on a 2m x 2mm glass column packed with 0.1% SP1000 on 80/100 mesh Carbopack C (column temperature 135°C , nitrogen carrier gas 30ml/min) and electron capture detection. Under these conditions TCE and ^{14}C -TRI were extracted quantitatively. TCE-glucuronide in the aqueous phases was hydrolysed by incubating with bacterial β -glucuronidase (100 units) for 2 hours at 37°C . The hydrolysed TCE was extracted with a further 2 x 4ml of diethyl ether and analysed by gas chromatography. In some experiments the pH of the aqueous phases was adjusted to 5.0, arylsulphatase (20 units) added, and incubated for 2 hours at 37°C . Extraction and analysis was then carried out as above. TCA was extracted from the aqueous phase with 6ml of diethyl ether after the addition of 2ml 1M sulphuric acid and quantified by liquid scintillation spectrometry.

2.5.3 Viability of Cells Exposed to TRI In Vitro: The viability of Clara cells exposed to TRI (0-10mM) was determined by trypan blue exclusion. The viability of hepatocytes during incubation with TRI was monitored by measuring lactate dehydrogenase (LDH) leakage into the medium. Cells were removed from the medium by centrifugation and LDH determined in the medium using a KONE analyser and a Boehringer test kit (Lewes, Sussex, UK).

2.5.4 Enzyme Assays: Protein was determined by the method of Bradford (1976) using reagent supplied by Biorad (Watford, Herts, UK). Ethoxycoumarin O-deethylase activity (ECOD) was determined by the method of Aitio (1978), aldrin epoxidation (AO) by the method of Wolff et al (1979) and NADPH cytochrome C reductase (CR) by the method of Williams and Kamin (1962). Glutathione S-transferase (GST) was determined with chlorodinitrobenzene as substrate (Habig et al, 1974) and UDP glucuronosyl transferase with the substrates 2-aminophenol (Dutton, 1980) and 4-nitrophenol (Sanchez and Tephly, 1974). Glucuronosyl transferase was determined without activation.

TCE glucuronidation in Clara cell sonicates, liver homogenates and liver microsomes were compared using equivalent amounts of protein (0.5mg) and incubated for 30 minutes at 37°C. Sealed vials contained 75mM Tris HCl pH 7.4, 10mM MgCl₂, 1mM TCE (1µl in DMF) and 0.5mg protein in a final volume of 1ml. The reaction was started by addition of UDP-glucuronic acid (3mM) and stopped by addition of ice-cold diethyl ether (4ml). Unconjugated TCE was removed by exhaustive extraction (4 times) with diethyl ether (4ml). TCE-glucuronide was then hydrolysed and quantified as described above.

3. RESULTS

3.1 The Effects of TRI on Mouse and Rat Lung

Exposure of mice and rats to TRI resulted in no significant clinical effects other than mild anaesthesia in mice exposed to the higher doses. There were no effects on lung/bodyweight ratios in either species (data not shown).

Light microscopic examination of lungs from mice given a single exposure of TRI revealed vacuolation of Clara cells at all dose levels (Figure 1). The number of Clara cells showing vacuolation was dose dependent. At 20ppm the majority of Clara cells were unaffected whereas at 200ppm most Clara cells had marked vacuolation. Pyknosis of the bronchiolar epithelium also occurred and was most severe at the higher dose levels. At 2000ppm, focal loss of the bronchiolar epithelium was evident with some exudate present in the lumen. The lesion was specific to the Clara cell at the light microscope level, no damage being apparent to other cell types.

After either 4 or 5 consecutive exposures to 450ppm of TRI the mouse lungs appeared morphologically normal. The Clara cell vacuolation evident after a single exposure had disappeared. The lesion reappeared when one further exposure was given after 2 days without exposure, and was more marked. After a further 4 or 5 exposures the mouse lungs again appeared morphologically normal (Figure 1).

Enzyme activities in Clara cell sonicates prepared from mice after a single exposure were unaffected at 20ppm, but at 100ppm and above cytochrome P-450 activities were reduced (Figure 2). ECOD activity decreased to 55% control at 200ppm and to 25% control at 2000ppm. AO showed a maximum decrease to 65% of the control value. CR was also reduced (35-55% control) whilst GST was generally unaffected. On multiple exposure to TRI, ECOD

activity remained reduced throughout the 10 exposures whilst A0 recovered to control levels, although was variable. CR also recovered gradually over the duration of the study. GST was again unaffected (Figure 3).

No compound related effects were observed in the lungs of rats exposed to 1000 or 500ppm of TRI. At the light microscope level all cell types appeared normal.

The effects of TRI on enzyme activities in rat lung microsomes and cytosol differed from those obtained with mice. Of the microsomal activities A0 was reduced to 54-71% control values, CR was also reduced (70% control) but ECOD was unaffected. Cytosolic GST was also unaffected (Table 1).

3.2 Isolated Clara Cells and the Metabolism of TRI

The viability of Clara cells incubated with DMF (1 μ l in 1ml of cell suspension) in sealed flasks remained high for at least 2 hours, declining only from 95% to 87% (Figure 4). TRI caused some slight loss of viability at concentrations of 5 and 10mM, the lowest viability being 65% after 2 hours.

TRI was metabolized primarily to chloral by isolated Clara cells. Levels of chloral reached a plateau after 60 minutes but still remained the major metabolite. TCE was formed more slowly giving levels which always remained lower than those of chloral. A small amount of TCA formation was also seen (Figure 5). TCE-glucuronide was not found. Hydrolysis with arylsulphatase indicated that TCE was not forming a sulphate conjugate in Clara cells in preference to the glucuronide. Incubation of TRI with Clara cells in a more complex medium (Medium 199, Gibco) rather than HBSS gave qualitatively and quantitatively identical metabolites. The lack of glucuronide formation did not therefore appear to be due to a deficiency either of cofactors or the analytical procedures. Authentic TCE-glucuronide (from mouse urine) added to incubations was readily detected.

3.3 Isolated Hepatocytes and the Metabolism of TRI

TRI at a concentration of 1mM was not toxic to mouse hepatocytes. The increase in LDH leakage which did occur during incubation of hepatocytes with TRI was similar to that seen in controls (Table 2).

The overall metabolism of TRI was greater in hepatocytes than in Clara cells and the metabolic profile was also qualitatively different (Figure 6). TCE was the major metabolite, after 2 hours levels were 17 fold higher than in Clara cells. TCE-glucuronide was also produced and after 2 hours incubation was the second most abundant metabolite. Chloral concentrations were up to 2 fold higher than in Clara cells whilst TCA increased to a level similar to that of chloral.

3.4 Comparison of Glucuronosyl Transferase Activity in Mouse Lung and Liver

Following the observation of an apparent deficiency in TCE-glucuronidation in the Clara cell, the activities of glucuronosyl transferase towards two phenolic substrates (2-aminophenol and 4-nitrophenol) and to TCE were compared in liver fractions and lung Clara cells. 2-Aminophenol and 4-nitrophenol glucuronidation in Clara cell sonicates were 2-3 times higher than in liver microsomes. TCE-glucuronidation, however, was barely detectable in Clara cell sonicates being 30 fold lower than in liver microsomes and 12 fold lower than in total liver homogenates (Table 3).

3.5 The Effects of Chloral, TCE and TCA on Mouse Lung

Exposure to 100ppm of chloral for 6 hours induced deep anaesthesia although on cessation of exposure the mice recovered normally. TCE also induced a deep anaesthesia. Exposure to 100ppm was continued for 6 hours whilst exposure to 500ppm was discontinued after 2 hours. After exposure all animals recovered normally. Mice dosed with TCA (200 or 500mg/kg, i.p.) showed no clinical abnormalities.

The lungs of mice exposed to chloral showed identical bronchiolar lesions to those seen with TRI. The effect with chloral was more severe, 100ppm of chloral producing an equivalent effect to 1000ppm of TRI. Exposure to chloral also resulted in some alveolar necrosis and desquamation of the epithelium. Alveolar oedema was also evident.

In contrast, TCE induced minimal Clara cell damage, 66% and 33% of animals exposed to 100 or 500ppm respectively of TCE were unaffected. In the remaining animals mild Clara cell vacuolation occurred but there were no alveolar lesions. TCA treatment produced no adverse morphological effects on the mouse lung.

The effects of chloral, TCE and TCA on Clara cell biochemistry are in accordance with the pathological observations (Table 4). Chloral increased lung/bodyweight ratios 1.5 fold, probably as a consequence of the alveolar oedema. CR was decreased to 71% control values whilst ECOD and GST were unaffected. TCE had no effect on any of these parameters at either exposure level. The only effect observed with TCA was a 1.6 fold increase in ECOD at the 500mg/kg dose level.

4. DISCUSSION

The acute and long term effects of TRI in laboratory animals have been extensively investigated (WHO, 1985, Crebelli and Carere, 1989). The hepatocarcinogenicity of TRI in the mouse has been shown to be associated with peroxisome proliferation (Elcombe *et al.*, 1985). This response has not been described in the lung, even with the more potent peroxisome proliferators. Furthermore, a study in this laboratory (Elcombe, unpublished) failed to find peroxisome proliferation in the lung following inhalation exposure of mice to TRI (450ppm 6 hours/day for 10 days). Thus, the only known effects of TRI on the mouse lung were those described by

Forkert et al (1985, 1989) following administration by the intraperitoneal route. An investigation of the Clara cell lesion following inhalational exposure, the effects of dose and repeated exposure, the mechanisms involved, and their relevance to other species formed the basis of the present work. Having characterised the acute lesion, its relevance to the development of lung tumours can then be considered.

A single inhalation exposure of mice to TRI induced specific damage to the lung Clara cell with other cell types being unaffected. The histopathological lesion and loss of cytochrome P-450 were very similar to those described after i.p. doses of TRI (Forkert et al, 1985). The reduction in cytochrome P-450 substrate activities in Clara cell sonicates may be partly due to general cell toxicity and partly a consequence of suicide inhibition of this enzyme. In the liver interaction of the oxidized TRI intermediate with the active site of cytochrome P-450 causes haeme degradation and loss of enzyme activity (Moslen et al 1977, Costa et al 1980). The loss of CR in Clara cell sonicates probably reflects cell toxicity, in a manner similar to that seen with the nitroblue tetrazolium reductase stain which is specific for Clara cells (Devereux and Fouts, 1980) and has been used to determine toxicity in cultured Clara cells (Richards et al 1990).

There was a clear species difference in the effects of TRI on rat lungs. No morphological effects were seen at doses which induced severe effects in mouse lungs. Some flattening of the bronchiolar epithelium has been reported in rats, but only after inhalation exposure to very high concentrations of TRI such as 8000ppm (Kurasawa, 1988). The biochemical effects in rats and mice are difficult to compare directly as in rats they were measured in whole lung microsomes rather than Clara cell sonicates. Nevertheless, the reduction of AO and loss of microsomal CR indicates that TRI is probably also causing some cytochrome P-450 degradation in the rat lung.

The recovery of the histopathological lesion, CR and AO activities on repeated exposure of mice to TRI, whilst ECOD remained reduced, has also been shown to occur with methylene chloride (Foster et al 1986, 1991) and naphthalene (Buckpitt and Franklin 1989). It has been suggested for these two compounds that the product of P-450 metabolism is responsible for the initial lesion and that the continued reduction in P-450 results in reduced metabolite formation and hence morphological recovery of the Clara cell. It is possible that a similar situation occurs with TRI. We have shown that the lesion recurs more severely on the first exposure after a rest period. If this pattern is repeated over the duration of a 2 year study, it may influence the development of tumours. However, at the present time it is not known whether damaged Clara cells recover, become necrotic or lead to increased cell division, factors which will determine their impact on carcinogenesis.

TRI was metabolized to chloral in mouse Clara cells at rates which compared well with those in hepatocytes. However, the metabolism of chloral to TCE and TCE glucuronide was either diminished, or in the latter case, virtually absent in Clara cells. The reduction of chloral to TCE in liver is catalysed by alcohol dehydrogenase (Friedman and Cooper 1960, Ogino et al 1990) which has low activity in lung (Sorokin, 1970). TCE-glucuronide was not formed during the metabolism of TRI in the isolated Clara cell and activity was barely detectable in Clara cell sonicates when TCE was used as a substrate. By comparison, the activity of UDP-glucuronosyl transferase towards the phenolic substrates was even higher in Clara cells than in liver fractions. This suggests that TCE is not a substrate for the 3-methyl cholanthrene inducible isoform of glucuronosyl transferase known to metabolize these substrates.

In the in vivo experiments chloral induced an identical bronchiolar lesion to TRI whereas TCE and TCA were ineffective. This clearly indicates that chloral is the metabolite responsible for TRI induced lung injury. The metabolic deficiencies in the metabolism of chloral in the Clara cell are thought to lead to the accumulation of chloral with resulting toxicity. Damage to the alveolar epithelium, but not to ciliated bronchiolar

epithelial cells may also reflect a difference in the enzymes needed to detoxify chloral. The noted specificity for Clara cells seen in the TRI experiments probably reflects the high cytochrome P-450 activity present in these cells for the metabolism of TRI to chloral. This high cytochrome P-450 activity in conjunction with the lack of alcohol dehydrogenase and glucuronosyl transferase activity results in the specific Clara cell lesion. Clara cells have also been suggested to be inherently more sensitive to xenobiotic insult than other cell types in the lung (Massey, 1989).

These studies have suggested a plausible explanation for the development of the morphological lesion and the biochemical changes seen in the mouse lung after exposure to trichloroethylene. There remains the question of the significance or relevance of these changes to the subsequent development of lung tumours. It is highly unlikely that acute studies of this nature can definitely establish the cell of origin of lung tumours which are observed for the first time at the end of a 2-year carcinogenicity bioassay. However, the present studies present evidence to suggest that these tumours may arise from Clara cells. Firstly, the effects of TRI are highly specific and are seen only in Clara cells, secondly, the species differences between mice and rats seen in the acute studies reflect those seen in the 2 year cancer studies and, thirdly, there are the known cytotoxic and genotoxic effects of chlorinated aldehydes of which chloral is a member. 2-Chloroacetaldehyde and 2,2-dichloroacetaldehyde are both alkylating agents which are mutagenic (Bignami et al 1980) and have been shown to covalently interact with cellular macromolecules (Guengerich et al 1977, Liebler et al 1985). Chloral (trichloroacetaldehyde) itself is mutagenic in the Ames bacterial mutation assay without requiring metabolic activation (Waskell 1978; Bruce and Heddle 1979; Bignami et al 1980) and has also been shown to cause disruption of the chromosome mitotic spindle resulting in aneuploidy (Kufer 1986; Miller and Adler 1989). Thus the high activating, but low detoxifying capacity of Clara cells towards trichloroethylene leads to the accumulation of a metabolite of known genotoxic potential, and in circumstances of repetitive cell damage that

result from the exposure regime commonly used in animal cancer bioassays. These observations form the basis of a potential mechanism which explains the basis of the lung tumours in mice and suggests that they are of Clara cell origin. Other organs, for example the liver, appear to be protected from the adverse effects of chloral by efficient detoxifying systems.

The mechanism appears to be restricted to the mouse, rat lungs being unaffected by exposure to trichloroethylene. In addition, Clara cells are less abundant in the rat lung (Plopper 1983) and the metabolism of TRI is significantly lower in the rat in vivo than in the mouse (Prout et al 1985). Clara cells are relatively few in number in human lung and are morphologically different from those in rodents. Smooth endoplasmic reticulum, and therefore presumably cytochrome P-450 activity, appears to be absent from the human Clara cell (Smith et al 1979) suggesting that little or no metabolism of trichloroethylene will occur in these cells. Since the lesion seen in the mouse results from the metabolism of TRI to chloral it appears highly unlikely that a similar lesion will develop in human Clara cells after exposure to TRI. Equally, if the lesion is linked to the development of lung tumours, it follows that TRI would not present a carcinogenic hazard to the human lung.

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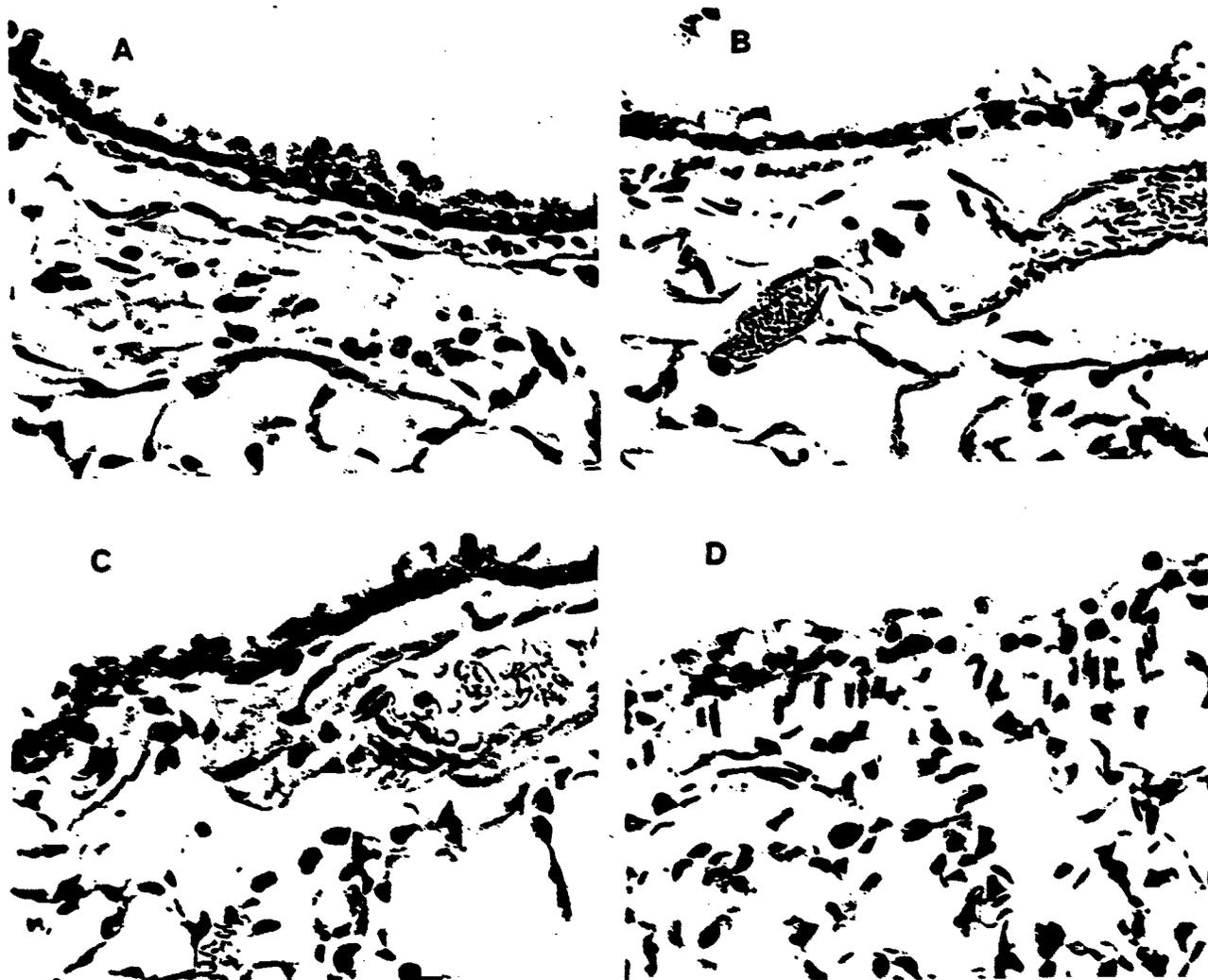
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A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

FIGURE 1

LIGHT MICROGRAPHS OF MOUSE LUNG BRONCHIOLAR EPITHELIUM

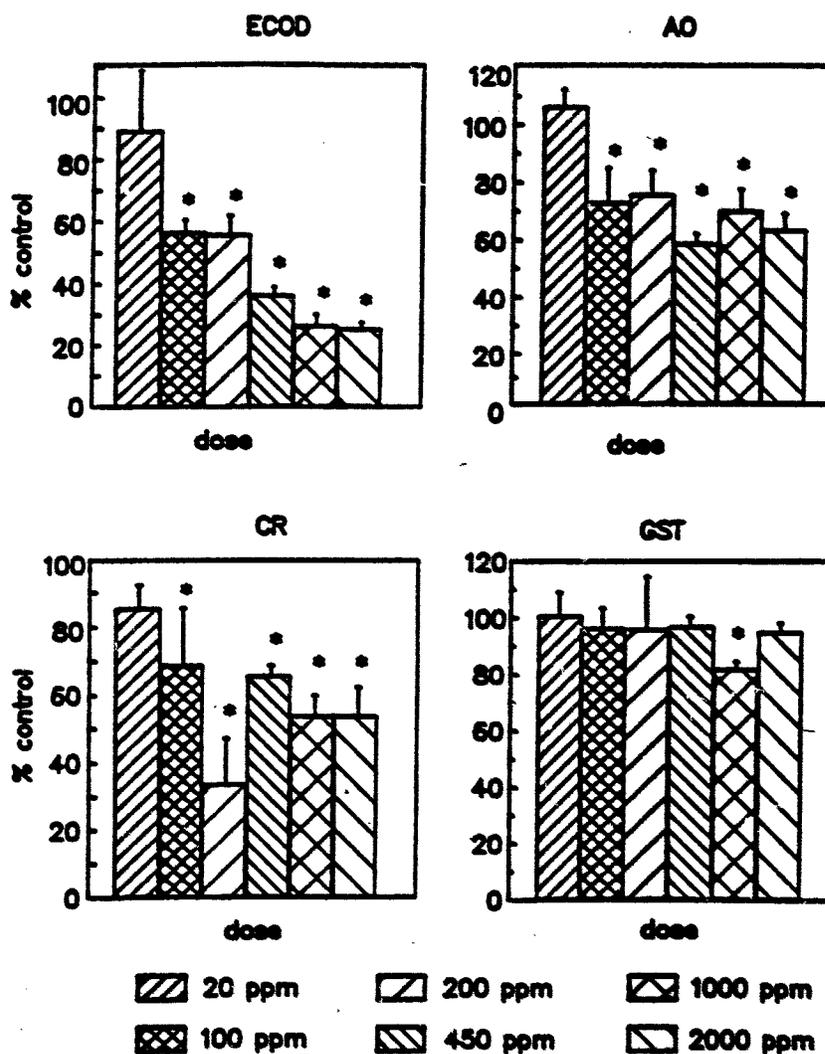


Clara cells are shown (arrow) protruding into the airway. A. Control. B. After a single exposure to TRI (450ppm for 6h) showing severe Clara cell vacuolation. C. After 5 consecutive exposures to TRI (450ppm for 6h/day) showing recovery of the lesion. D. After a sixth exposure to TRI (as C) following 5 days exposure, 2 days break showing recurrence of the lesion.

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FIGURE 2

ENZYME ACTIVITIES IN CLARA CELLS ISOLATED FROM MICE
GIVEN A SINGLE EXPOSURE TO TRI

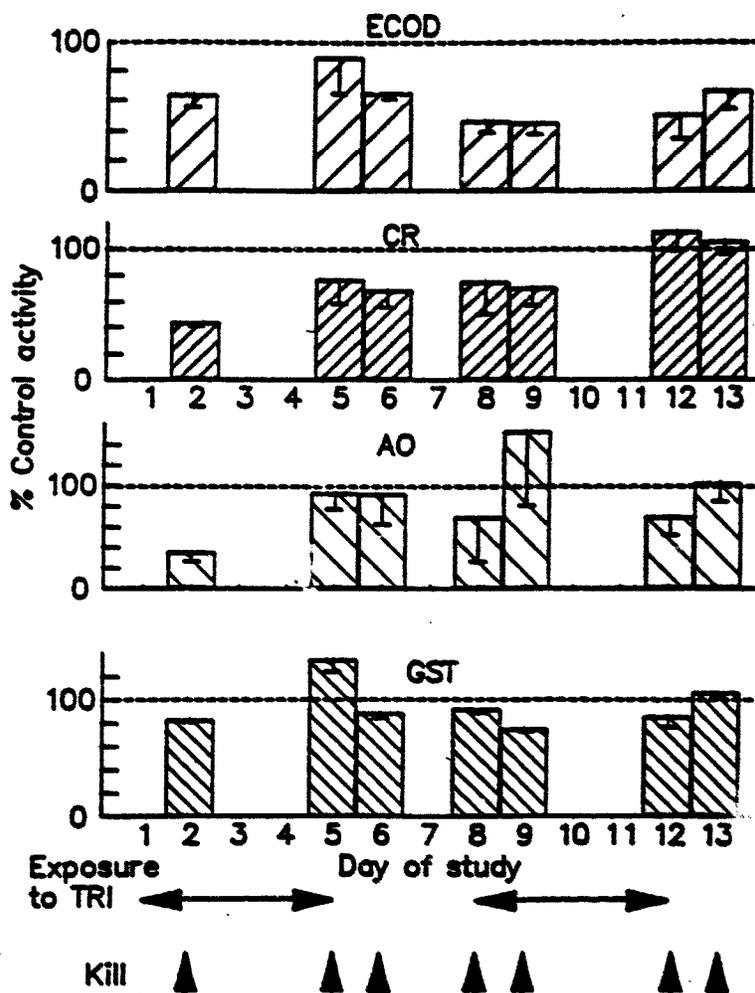


Values are mean \pm s.d. of % control values of 3 determinations using cells pooled from 6 mice. * Statistically significant $P < 0.05$. Actual control activities were: ECOD 100-150pmol/min/mg, AO 100-200pmol/min/mg, CR 120-200nmol/min/mg, GST 900-1200nmol/min/mg.

A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
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FIGURE 3

ENZYME ACTIVITIES IN CLARA CELLS ISOLATED FROM MICE EXPOSED TO
TRI FOR 2 WEEKS (450ppm 6h/day, 5 days/week)



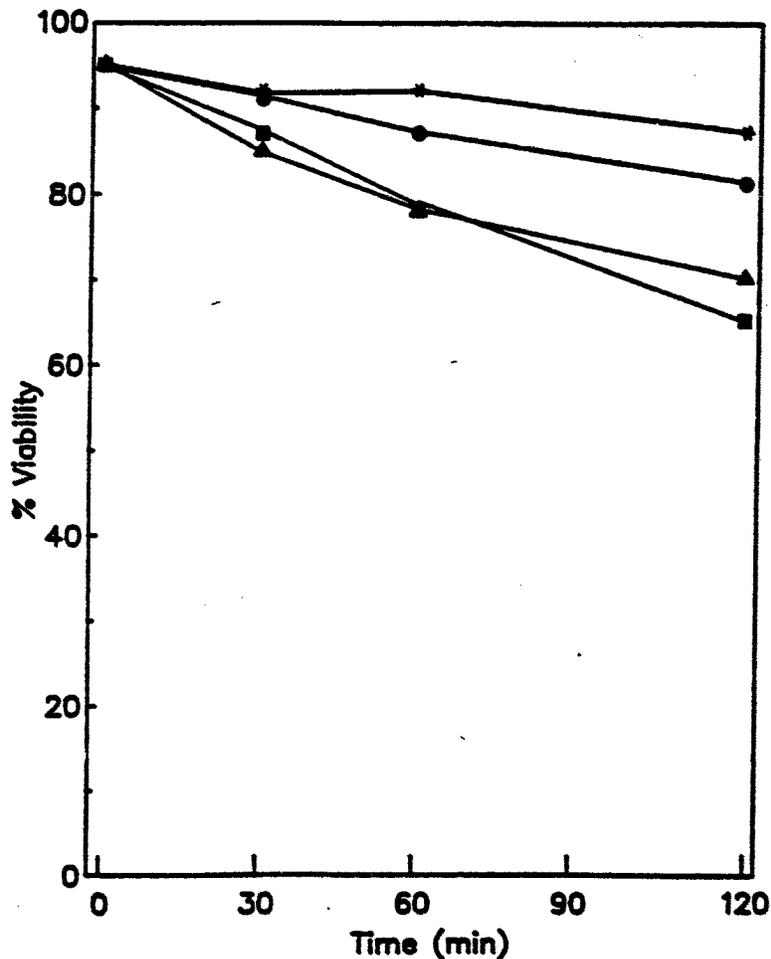
Values are mean \pm s.d. of % control values of 3 determinations using cells pooled from 6 mice. * Statistically significant $P < 0.05$.

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A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

FIGURE 4

VIABILITY OF MOUSE CLARA CELLS INCUBATED WITH TRI IN VITRO



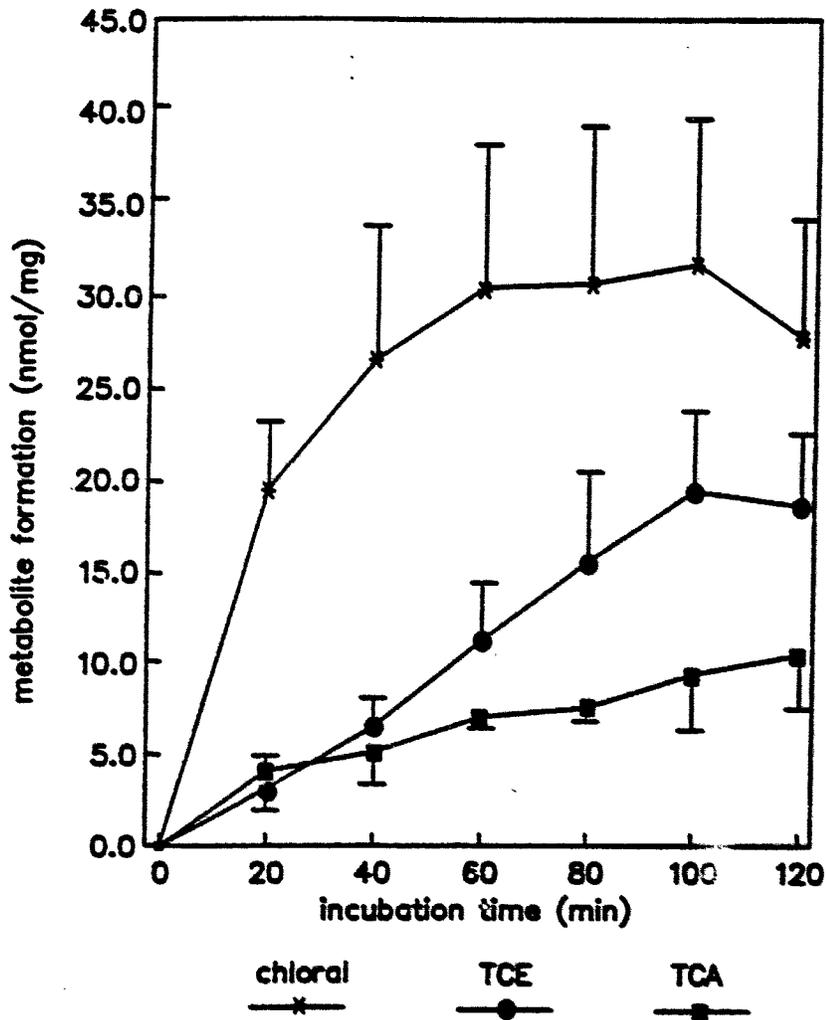
(*) DMF control, (●) 1mM, (■) 5mM, (▲) 10mM TRI. Viability was assessed by trypan blue exclusion. Values are means (n=4), standard deviations were all 2-5% and for clarity are not shown.

0.036

A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

FIGURE 5

THE METABOLISM OF TRI IN MOUSE CLARA CELLS



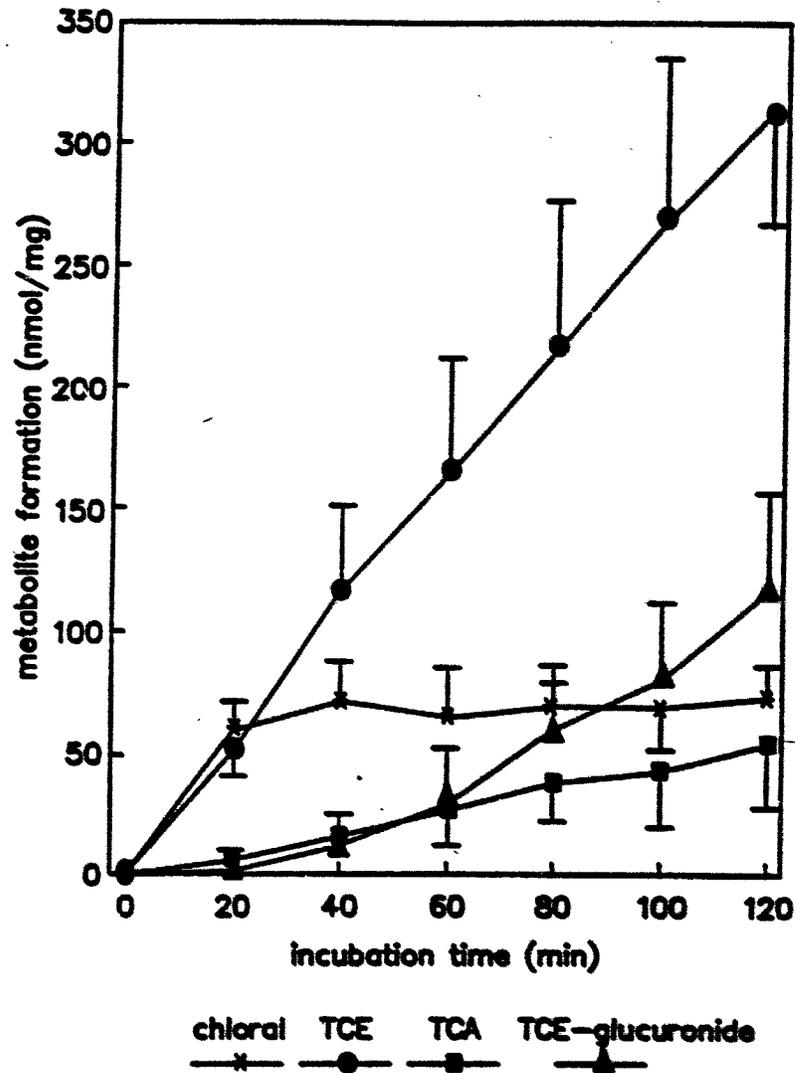
TRI (1mM) was incubated with 1×10^6 cells in 1ml. Values are mean \pm s.d., n=6.

0 10 3 7

A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

FIGURE 6

THE METABOLISM OF TRI IN MOUSE HEPATOCYTES



TRI (1mM) was incubated with 1×10^6 cells in 1ml. Values are mean \pm s.d., n=6.

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A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

TABLE 1

THE EFFECT OF TRICHLOROETHYLENE ON RAT LUNG

Assay	Control	TRI 500ppm	TRI 1000ppm
ECOD (pmol/min/mg)	64.8 ^a ±19.8	56.0 ±18.0	60.5 ±20.4
AO (pmol/min/mg)	147.5 ±32.5	80.0* ±20.0	105.0* ±22.5
CR (nmol/min/mg)	50.1 ±6.4	33.7* ±3.8	38.9* ±4.5
GST (nmol/min/mg)	111.4 ±20.8	133.0 ±14.6	114.6 ±48.6

Animals were exposed to TRI for 6 hours, controls were exposed to air only.

^a Values are mean ± s.d., n=3

* Statistically significant P<0.05

A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

TABLE 2

LACTATE DEHYDROGENASE LEAKAGE FROM MOUSE HEPATOCYTES
INCUBATED WITH TRICHLOROETHYLENE

Incubation Time (min)	Control	TRI (1mM)
0	440 ± 55 ^a	421 ± 28
30	537 ± 94	593 ± 137
60	581 ± 112	585 ± 48
120	727 ± 168	736 ± 128

^a Values are expressed in IU/litre for 1×10^6 cells incubated in 1ml.
Mean ± s.d. (n=4)

A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

TABLE 3

A COMPARISON OF UDP-GLUCURONOSYL TRANSFERASE
ACTIVITIES IN MOUSE LIVER AND LUNG

Substrate	Liver microsomes	Liver homogenates	Clara cell sonicates
2-Aminophenol	0.319 ±0.044	0.189 ±0.028	0.967 ±0.234
4-Nitrophenol	3.317 ±0.532	0.822 ±0.073	6.283 ±2.819
Trichloroethanol	0.510 ±0.152	0.205 ±0.047	0.017 ±0.004

Values are nmol/min/mg, mean ± s.d.

Livers n=6, lungs n=3

A MECHANISM FOR THE DEVELOPMENT OF CLARA CELL LESIONS
IN THE MOUSE LUNG AFTER EXPOSURE TO TRICHLOROETHYLENE

TABLE 4

THE EFFECT OF TRICHLOROETHYLENE METABOLITES ON MOUSE CLARA CELLS

Compound/ Dose Level	Lung/ Bodyweight	ECOD	CR	GST
Chloral 100ppm, 6h	149 ± 7.4 ^a	102 ± 28.9 ^b	71 ± 5.1 ^b	157 ± 54.2 ^b
TCE 100ppm, 6h	96 ± 8.2	118 ± 26.5	91 ± 7.1	98 ± 5.5
500ppm, 6h	109 ± 12.9	85 ± 21.2	96 ± 3.7	106 ± 9.1
TCA 200mg/kg i.p.	- ^c	111 ± 38.6	101 ± 5.9	105 ± 22.2
500mg/kg i.p.	-	157 ± 39.1	94 ± 7.1	111 ± 2.0

^a Values are mean ± s.d. of % control values of 3 determinations using cells pooled from 4 mice.

^b Control enzyme activities are in the ranges quoted in Figure 2

^c - not determined

* Statistically significant P<0.05

0 0 4 2