TCE - Cancer

CHEMICAL MANUFACTURERS ASSOCIATION

September 9, 1980

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To: Trichloroethylene Project Panel

Gentlemen:

Enclosed for your information is your copy of a report entitled: Trichloroethylene: Molecular and Biological Aspects of Its Mutagenic and Carcinogenic Potential. CMA received this document on September 4, 1980 from the European Chemical Industry Ecology and Toxicology Center, Avenue Louise 250, Bte 63, 1050 Brussels.

Sincerely,

J. T. Seawell
Project Administrator
Trichloroethylene

JTS:md
Enclosure

SL 034718

FONDAZIONE CARLO ERBA

SEZIONE: MEDICINA DEL LAVORO E IGIENE AMBIENTALE

20122 MILANO - VIA CINO DEL DUCA, 8 - TELEF. 02/780.469

TRICHLOROETHYLENE: MOLECULAR AND BIOLOGICAL ASPECTS OF ITS MUTAGENIC AND CARCINOGENIC POTENTIAL

Conferenza del

Professor DIETRICH HENSCHLER

Direttore dell'Istituto di Farmacologia e Tosssicologia

dell'Università di Würzburg, Repubblica Federale Tedesca

tenuta alla Fondazione Carlo Erba il 3 dicembre 1979

Subsequent to definite identification of vinyl chloride as a carcinogenic substance, both by animal experimentation performed by the Italian groups of Viola and Maltoni, as well as by findings of undisputed human liver-haemangiosarcomas following high and prolonged occupational exposure, the question was raised by several groups of research workers as to whether this unique and deleterious effect is encountered with vinyl chloride alone, or as well with the other members of the chemical family of chlorinated ethylenes. Among these, trichloroethylene is the most prominent, not only by virtue of the production figures world-wide but also, or even more so, by the amount and frequency of exposure in occupational as well as in non-industrial areas. Trichloroethylene has been used as a general anaesthetic in surgery and obstetrics, and addictive states of narcosis have been produced only too often ("sniffing").

Table 1

We ourselves were immediately suspicious of a carcinogenic potential of trichloroethylene after the first report on the incrimination of vinyl chloride. Our motive was, however, different from that of other groups. We had performed extensive studies on the pharmacokinetics and metabolism of trichloroethylene in volunteers, in order to create a scientific basis for measuring exposure of occupationally exposed people by

biological monitoring. Let me start my presentation with the results of this work because it is relevant to the mechanistic evaluation of the carcinogenic potential of trichloroethylene.

It has been long suspected, and confirmed by a variety of more recent animal experiments, that trichloroethylene (tri) is, in most of its acute and chronic toxic effects, not active per se, but must undergo metabolic activation to chemically reactive intermediates. The following scheme for the metabolic

Fig. 1

transformation of tri has been valid for decades: trichloroethylene is converted to an instable epoxide which rearranges to chloral (or chloral hydrate), which then is transformed to trichloroethanol and trichloroacetic acid. The latter is excreted unchanged; trichloroethanol mostly after glucuronidation.

We then looked into the balance of the reaction, and the suitability of the metabolites for monitoring procedures in blood and urine. Groups of 5-6 volunteers were exposed 6 h/day, 5 days/week, for one or two weeks, to steady state concentrations of trichloroethylene of 50, 100, and 200 ppm. Metabolites were identified, and quantitatively determined by proper gas chromatographic methods.

Fig. 2

As can be seen from the following graph, the formation of the two main metabolites, as well as the pattern of excretion, differs extremely in that trichloroethanol (TCE) is formed immediately and dependent on the actual concentration of tri, and is excreted rather rapidly mostly in the form of its

glucuronide. Quite contrary to this, trichloroacetic acid (TCA) is formed very slowly, the formation going on for many hours after the end of tri exposure and the elimination proceeding extremely slowly; the half-lives being 12 hours for TCE, and 100 hours for TCA.

If we look at the pattern after consecutive daily exposure, we find a tremendous accumulation of TCA from day to day up to levels, at 50 ppm tri, of approximately 55 µg/ml, whereas with TCE there is no accumulation at all. After two weeks of exposure to 100 ppm tri, TCA accumulation comes to a steady state level of 115 µg/ml. This extremely high concentration of a foreign compound should cause considerable concern. Moreover, when checking the balance of metabolic transformation of tri by determining the cumulative excretion of the metabolites, one encounters a deficit of 40 to 50% which cannot be accounted for by the presently identified metabolites.

Fig. 3

Fig. 4

Right at that point of discussion we were confronted with the hypothesis that the carcinogenic effect of vinyl chloride could be attributed to the metabolic formation of its epoxide. We therefore immediately started a series of experiments on the epoxidation of all chlorinated ethylenes, and the formation of metabolites in vivo. The epoxides were synthesized, the rearrangement products identified, and the metabolites as found in vivo compared with the theoretically expected compounds. There was confirmation with all but one, the exception being trichloroethylene. The epoxide of this compound rearranges thermally in vitro to dichloroacetylchloride, whereas in vivo

Fig. 5

Fig. 6

Fig. 7

not a trace of dichloroacetic acid, the hydrolysis product of this, can be detected. The only metabolites found are TCE and TCA, both follow-up products of chloral.

What could be the reason for this discrepancy? We began a theoretical consideration of the mechanism of the intramolecular rearrangement of tri epoxide. Three possibilities exist: a simple hydride shift which, for a variety of reasons, is unlikely to occur; and C-O heterolyses which could occur in two positions. In both cases carbenium ions are to be expected as intermediates. If the heterolysis occurs in the position near the single chlorine substitution, the ionized carbon represents a more stable fragment than in the case of a twin chlorine substitution; thus, the formation of dichloroacetylchloride has a higher probability. In order to force the rearrangement the other way for chloral formation, an electron input is required. This speculation led us to try the involvement of Lewis acids as catalysts. Indeed, $FeCl_3$, or $AlCl_3$ readily induce the formation of chloral from tri epoxide, the attack possibly taking place at the oxygen, or the single chlorine, or at both.

Fig. 8

Fig. 9

Now, for our further consideration it is of key importance that the organism can provide such a Lewis-acid-like catalyst, precisely at the site of the formation of the epoxide. This is the haeme iron in the catalytic centre of P 450. From this, we set up the hypothesis that this trivalent iron could, within the hydrophobic premise of the enzyme, induce the rearrangement of the formed epoxide completely to the non-reactive chloral

so that no portion of this reactive intermediate is left with a chance to escape for covalent binding with macromolecules of the genetic material. Remember that the detection of only non-reactive metabolites is consistent with this hypothesis.

Tab. 2

To test further the validity of our assumptions, we carried out investigations on the behaviour of the epoxide under physiological conditions, i.e. in buffer solutions. Much to our surprise, the epoxide decomposes extremely rapidly mostly to smaller molecules. Firstly, all chlorine atoms are split off to form HCl. Secondly, carbon monoxide and formic acid are formed so that one obtains four equivalents of acid, i.e. three HCl and one formate. Under alkaline conditions, the yield of CO and HCOOH amounts to almost 100%. From these findings, the following mechanism of the C-C break has been proposed: the first step is the hydrolytic formation of a diol. Geminal substitution of one carbon atom with OH and Cl is, as has been demonstrated with the methylene halides, very instable and leads to rapid elimination of HCl. This process would render glyoxylic acid chloride, the hydrolysis product of which in fact has been detected by GC-MS techniques as a minor reaction product. The main pathway is, however, a C-C break. Why this occurs at all is unclear at present. However, we do have an explanation at hand of what goes on after the break has taken place. The fission products, formyl chloride and dichlorohydroxymethane, both render spontaneously CO, formate and HCl.

Fig. 10

If this decay of the epoxide would also occur in vivo, one should expect the formation of carbon monoxide haemoglobin. We have tested this. Groups of 10 rats were exposed, for 6 hrs., to 1000 or 2000 ppm tri, and blood samples analysed for carboxyhaemoglobin by a most sensitive GC method. As can be seen from the next graph, there is no CO formation under normal conditions. If we go back to our hypothesis, this has been proven valid so far, as any escape of the epoxide from the catalytic centre of P450 would have produced some detectable CO. There is, however, a very slight increase in the normal CO level after vigorous induction of P450 by pretreatment with high doses of phenobarbital. The significance of this finding is uncertain; it could indicate that in the state of strong enzyme induction a small proportion of tri epoxide leaves the hydrophobic premise and possibly finds access to targets susceptible for mutagenic and carcinogenic primary lesions. The consequences of this must be defined, of course, for practical conditions of human exposure.

Fig. 11

Another experiment has provided additional support for our hypothesis. This regards the capability of haeme iron to catalyze the rearrangement of tri epoxide to chloral. We used a haeme derivative, dimethoxy-protoporphyrin, with p-nitrophenyl sulphide as a fifth ligand to the iron. Under certain conditions which are most delicate because of the instability of the epoxide as well as the low solubility of the haeme complex, one gets a five to tenfold yield of chloral, as compared to the spontaneous rearrangement without the catalyst.

Fig. 12

Fig. 13

So far, we have a variety of indications that tri might be non-mutagenic and non-carcinogenic due to a potent detoxication mechanism which converts the highly reactive epoxide entirely to the non-reactive chloral. Indeed, most mutagenicity tests performed so far have been negative or questionable at the most. As the more interested among you might be aware, however, there is a report from the National Cancer Institute, Washington, D.C., on a carcinogenic effect of trichloroethylene in a special strain of mice. They observed, after daily gavage of extremely high doses, an increase of hepatocellular carcinomas in both sexes; such tumors occur with a relatively high spontaneous rate in this particular strain.

Tab. 3

We have checked the same sample of technical trichloroethylene as had been used in the NCI study and found, much to our surprise, by careful GC-MS analysis, a rather wide spectrum of contaminants, among these epoxides such as epichlorohydrin and epoxybutane, which have been demonstrated mutagenic by our group as well as by others. It took us a while to trace, in the older literature, the origin of these compounds in technical tri. Epoxides have been used as stabilizers, in conjunction with carboxylic acid esters. We have concluded from this that the NCI study might have been positive only due to these stabilizers, and not by virtue of trichloroethylene itself. This has been confirmed, to some extent, by Professor Loprieno of Pisa University who performed, based on our report, various mutagenicity tests in vitro and in vivo: pure or amine base stabilized trichloroethylene proved to be negative.

Fig. 14

Final proof for the anticipated non-carcinogenicity can only be based on a whole animal study with trichloroethylene, which should be of course stabilized by harmless compounds, not with carcinogenic epoxides. We performed such a life-time study in three animal species: mice, rats, and Syrian hamsters. Exposure was in individual wire cages, to 0 ppm (controls), 100 ppm and 500 ppm, 6 h/day, 5 days/week, for 18 months. The animals were kept until spontaneous death; thereafter they were autopsied, and underwent a careful histological investigation.

Tab. 4

Fig. 15

The results are, to cut a long story short, as follows: there is no increase in tumor formation in any sex, species or dosing group, with one exception: in female mice there is a dose-dependent increase in the formation of malignant lymphomas; 9/29 in controls, 17/30 in the 100 ppm group, and 18/28 in the 500 ppm group.

This type of tumor is, according to many reports in the literature, due to specific viruses which are inborn in certain strains of mice. They may be activated by carcinogens but also by non-specific stimuli. Such a non-specific stimulus might be seen in the derangement of certain hormone mechanisms by the accumulated TCA, which interferes with the protein binding of many substances; in derangements of metabolic processes, and others. In essence, the increase of this spontaneous tumor is not indicative of a carcinogenic potential of trichloroethylene.

May I conclude in stating that up to now there is convincing evidence both by whole animal experiments and by a variety of chemical and biochemical investigations that trichloroethylene is, under normal exposure conditions, neither a mutagen nor a carcinogen. The aforementioned bioassay performed by the NCI is most probably erroneous. Technical samples of trichloroethylene should, however, be carefully checked for carcinogenic impurities like vinylidene chloride and dichloroacetylene, and for stabilizers which bear this particular risk.

Table 1
World Production of Chlorinated Aliphatic Hydrocarbons 1973[1]

Compound	Formula	10^3 tons
1.2-Dichloroethane	CH_2Cl-CH_2Cl	19,500
Vinyl chloride	CH_2=$CHCl$	10,500
Perchloroethylene	CCl_2=CCl_2	1,050
Trichloroethylene	$CHCl$=CCl_2	1,010
1.1.1-Trichloroethane	CCl_3-CH_3	480
Carbon tetrachloride	CCl_4	1,000
Methylene chloride	CH_2Cl_2	400
Methyl chloride	CH_3Cl	350
Chloroform	$CHCl_3$	245

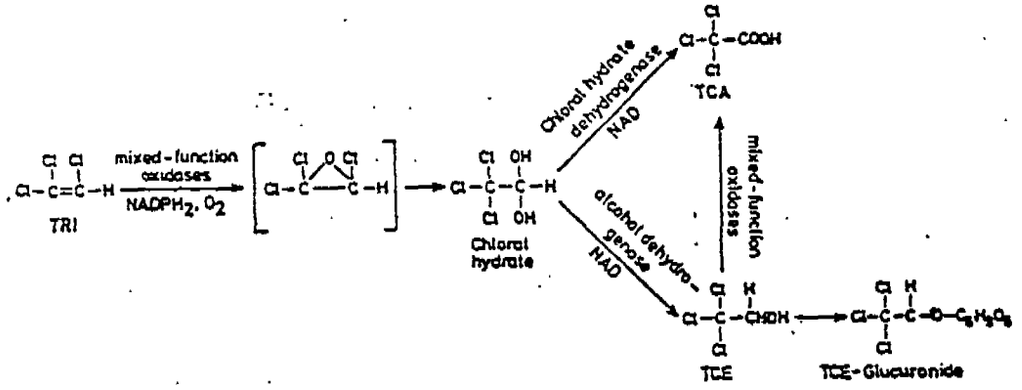

Fig. 1

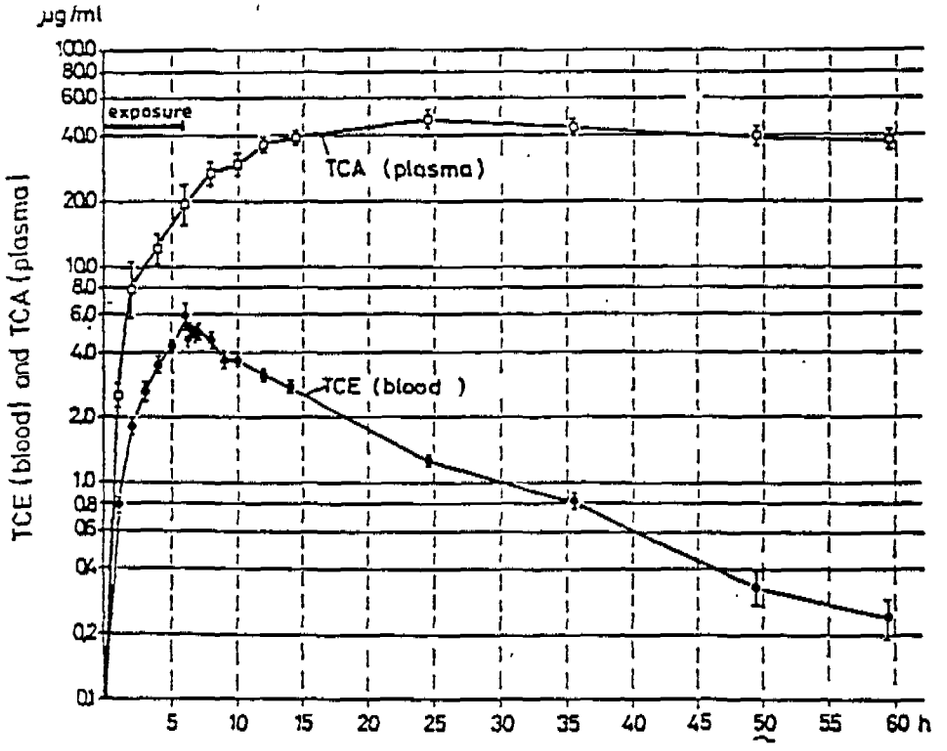

Fig. 2 Five-hour exposure of five volunteers to 100 ppm trichloroethylene. Levels of trichloroethanol (TCE, free form) in blood and of trichloracetic acid (TCA) in plasma.

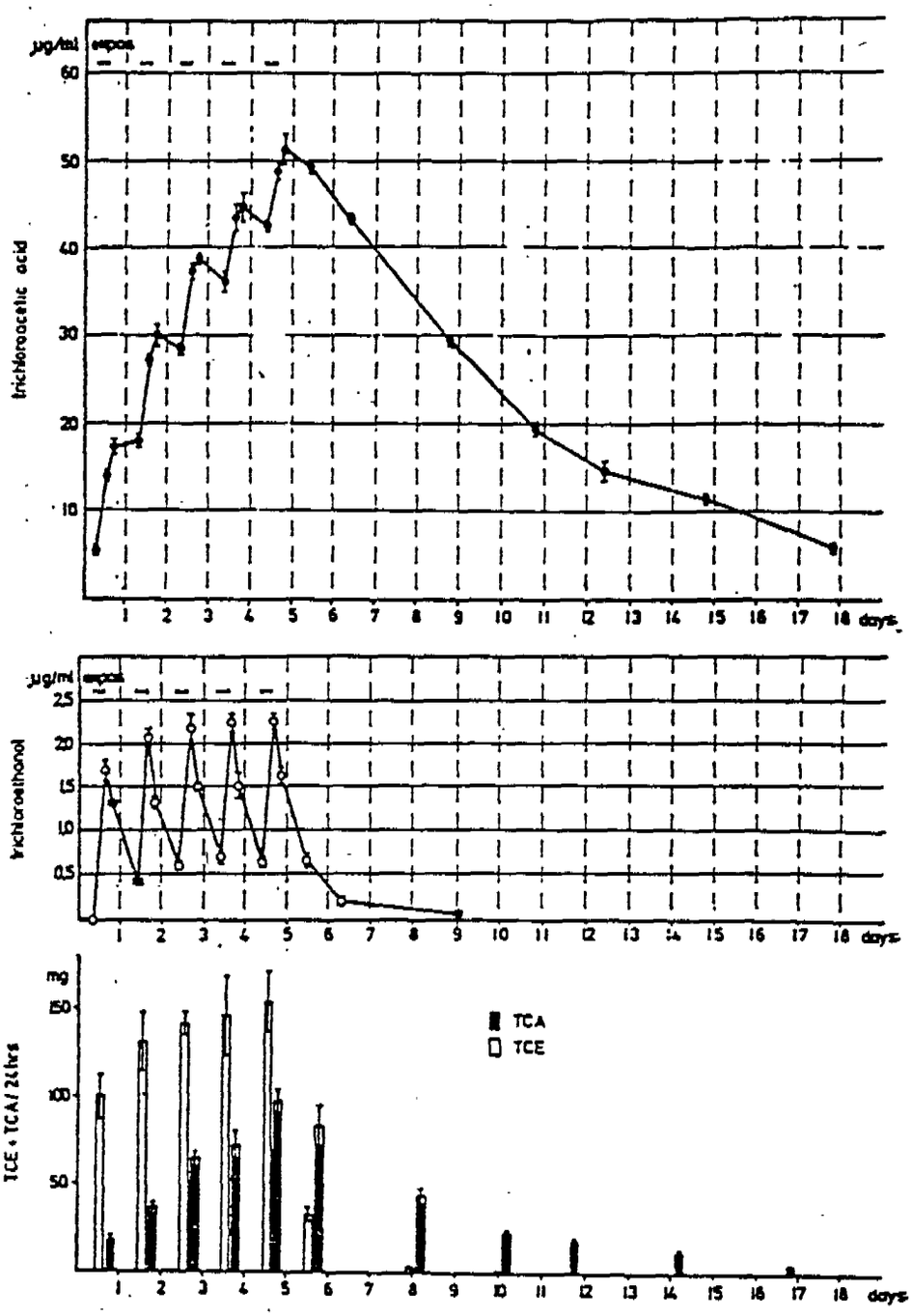

Fig. 3. Five days inhalation of trichloroethylene 50 ppm in five volunteers, 6 h/day. Upper part: levels of trichloroacetic acid (TCA) in plasma and trichloroethanol (TCE) in whole blood (free form).—Lower part: Urinary excretion of TCA and TCE (total = free and glucuronidized), 24 h-samples.

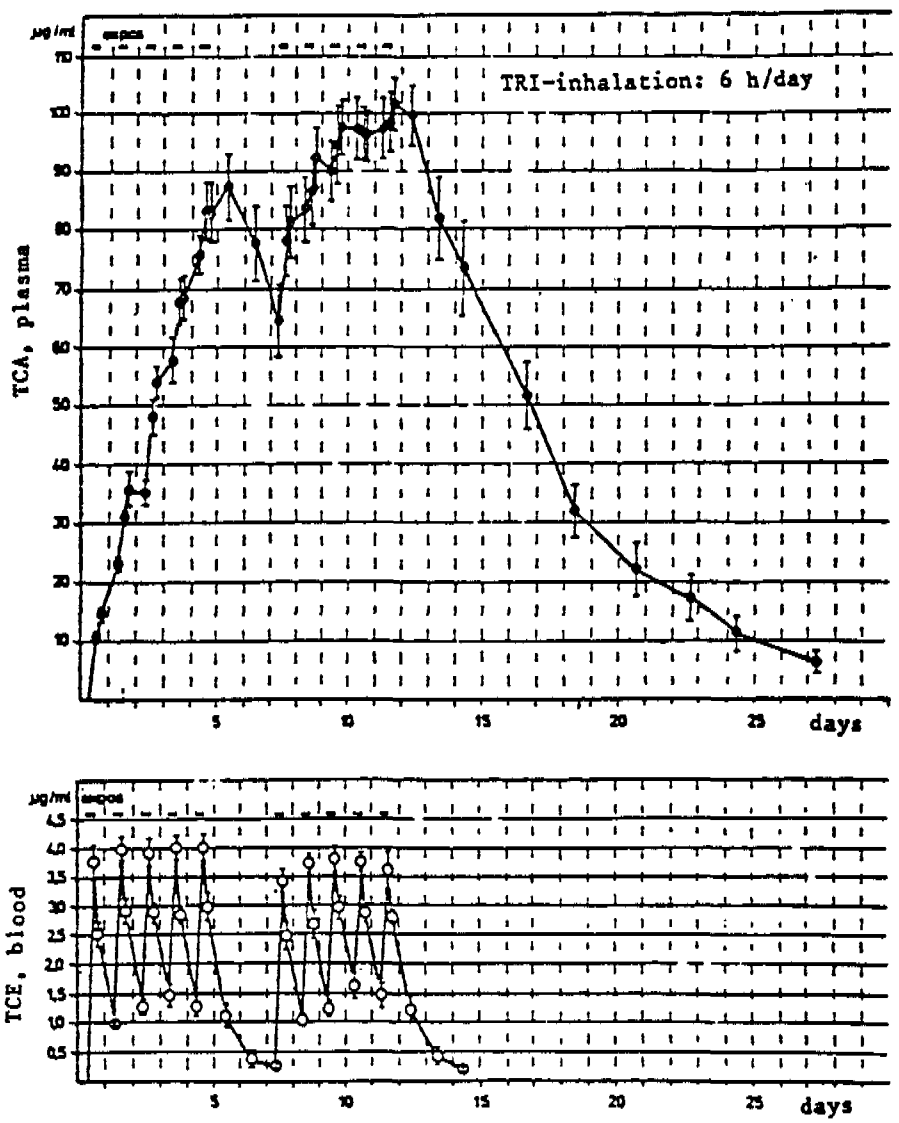

Fig. 4 Accumulation of trichloroacetic acid (TCA) in blood after two weeks, inhalation of 100 ppm trichloroethylene (5 days/week, 5 volunteers).

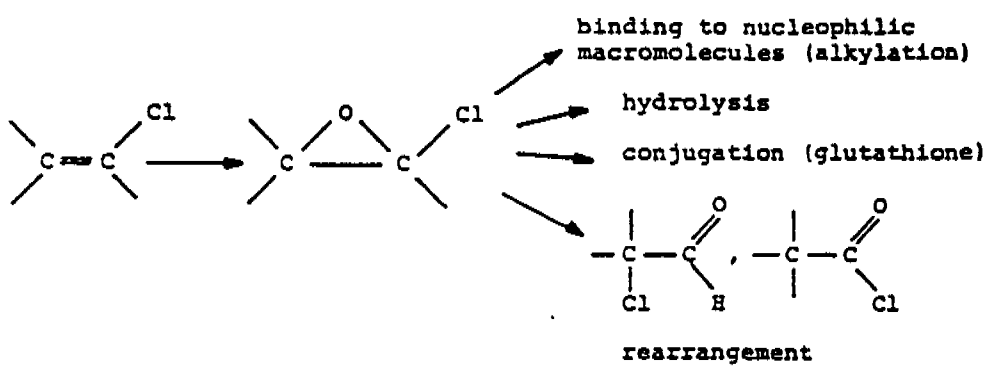

Fig. 5

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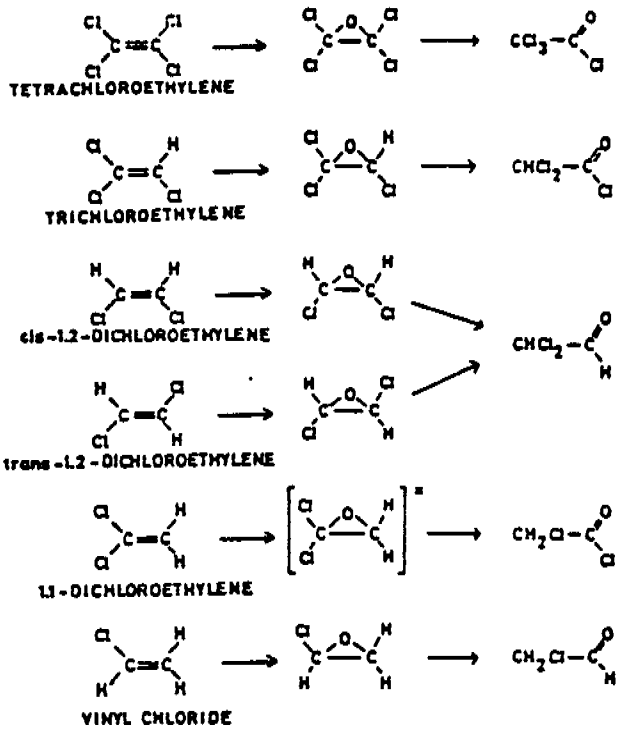


Fig. 6. Epoxidation of chlorinated ethylenes, and thermal rearrangement to either chlorinated aldehydes or acylchlorides. The epoxide of vinylidene chloride has not been synthesized up to now

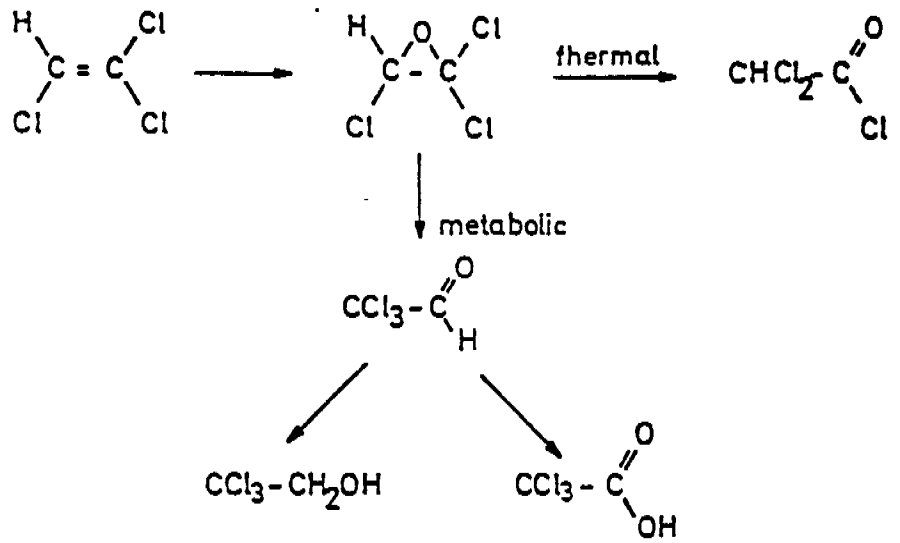


Figure 7 Different rearrangements of trichloroethylene oxide *in vitro* (to dichloroacetyl chloride) and *in vivo* (to trichloroacetaldehyde, chloral), and further metabolic conversion to trichloroethanol and trichloroacetic acid, respectively.

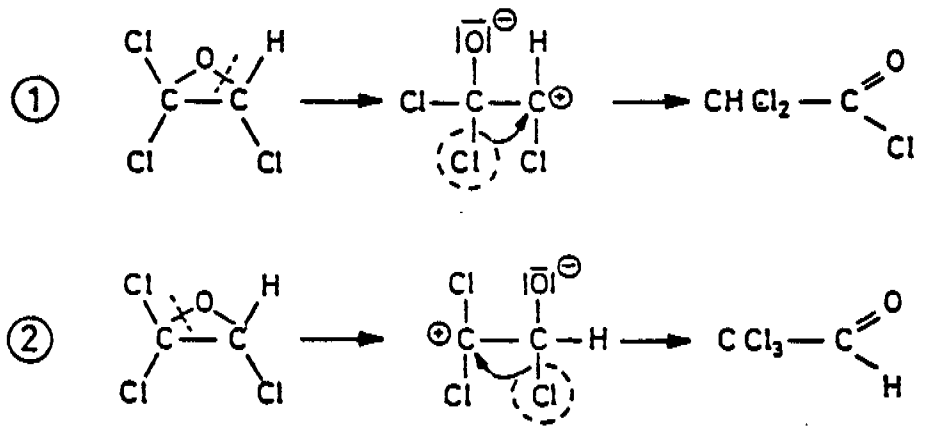


Figure 8 Possible mechanisms of rearrangement of trichloroethylene oxide, assuming ketocarbenium ions as intermediates after C-O heterolysis as a first step. (1) Simple chlorine substitution at the ionized

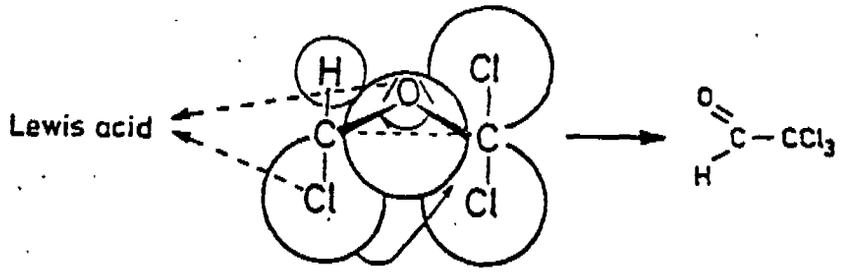
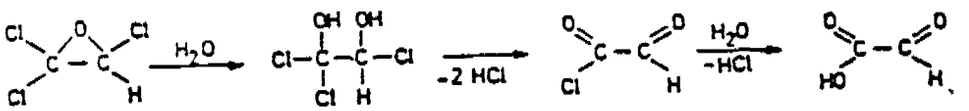


Fig. 9

Tab. 2: Formation of carbon monoxide, formate and dichloroacetic acid from trichloroethylene epoxide under varying pH in aqueous systems. Incubation mixture: 100 µM in 10 ml solution, vigorously shaken, left overnight at 4 °C. n=number of determinations

Medium	M	pH start	pH end	products identified (% theory of C_2HCl_3O) CO	HCOOH	n	$CHCl_2$–COOH	n
H_2O	-		1.8	16.0±1.3	16.5±2.1	5	3.4±1.3	3
KOH	0.1	13.0	12.8	47.8±9.3	46.5±2.4	6	6.25±1.7	3
NaOH	1.0	14.0	13.6	49.0±9.6	46.9±4.1	7	15.3±2.4	3
Tris-HCl	0.5	9.0	8.9	35.3±4.9	17.9±3.2	3	13.8±1.7	3
Tris-HCl	0.5	7.4	7.2	27.7±2.0	15.2±1.1	8	24.8±1.7	3
HCl	0.1	1.0	1.1	14.8±1.1	12.5±1.6	4	13.5±2.4	3
HCl	1.0	0	0.2	6.3±0.75	6.9±1.7	4	29.4±5.7	3



The resulting glyoxylic acid chloride will, in a final step, hydrolyse to the free acid.

The formation of carbon monoxide and formate may be formulated as follows:

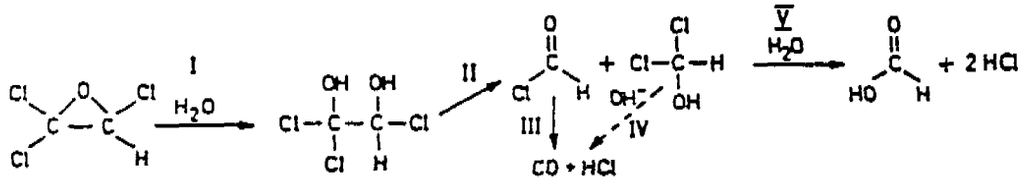


Fig. 10

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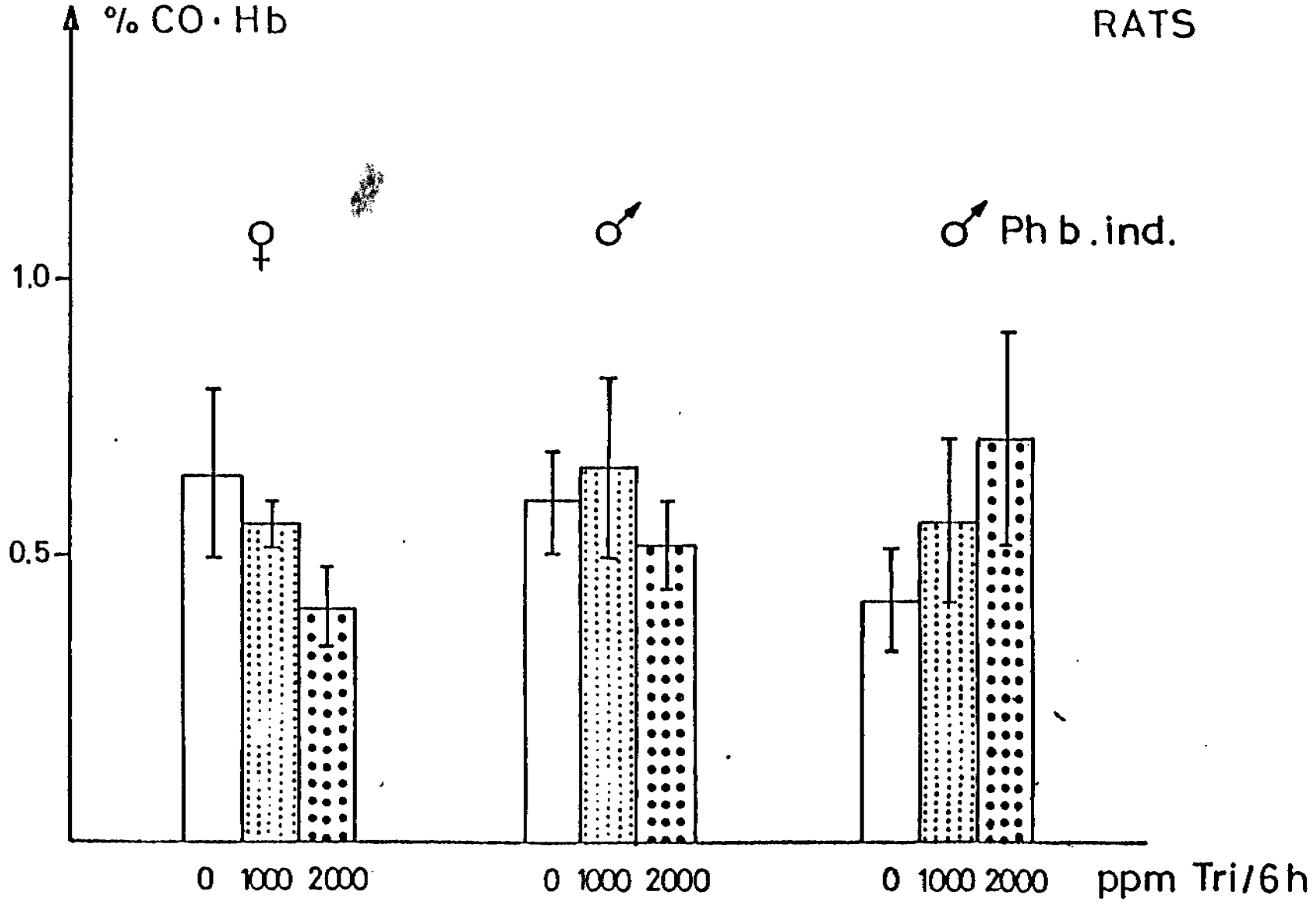

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Fig 11

Fe(PPIXDME)(SC_6H_4-p-NO_2)

Iron (III) Protoporphyrin IX Dimethyl Ester p-Nitrobenzenethiolate

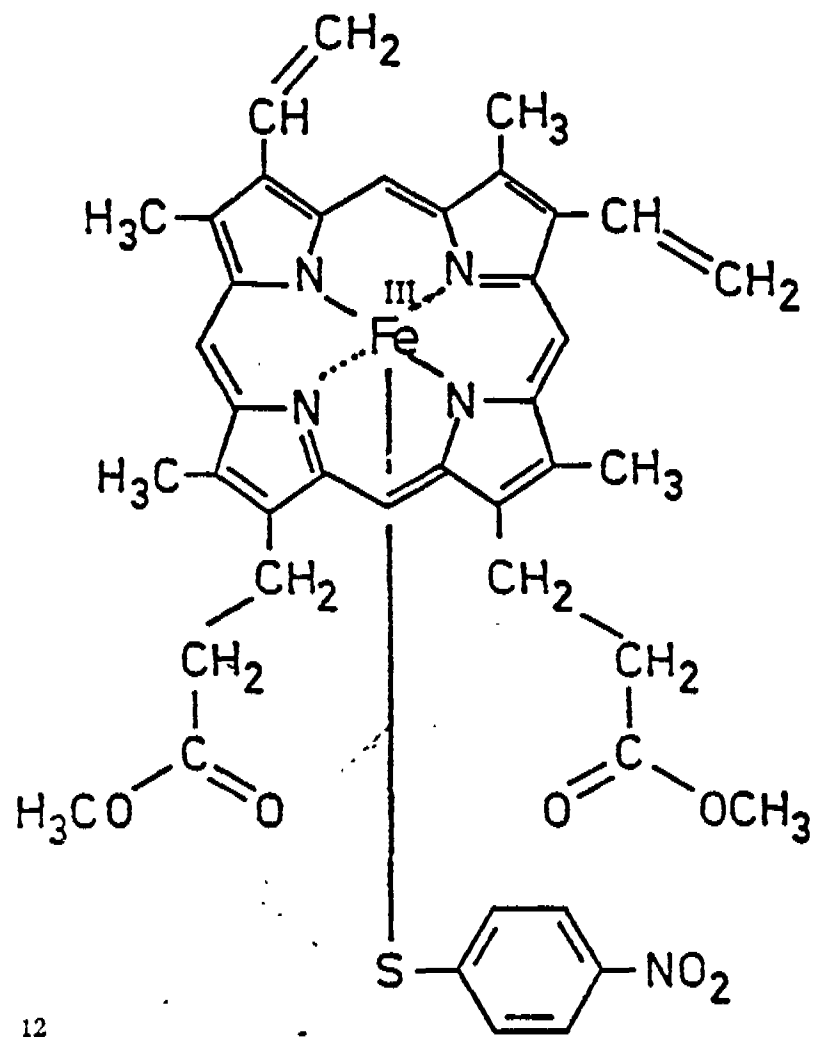

Fig. 12

REARRANGEMENT OF TRICHLOROETHYLENE EPOXIDE TO CHLORAL (50μMOLES, CYCLOHEXANE, -80 → 25 °C, 2 DAYS)

CATALYSATOR	-	$FeCl_3$		DMPP
μMOLES		2	6	1.3
% CHLORAL	0.2	1.7	5.8	1.4

Fig. 13

Table 3 Contaminants of a technical grade sample of trichloroethylene as identified by GC-MS. The typical mass fragments were completely identical for the fractions in the sample and a.r. grade samples for comparison

Compound	Main mass peaks m/e					
	% w/w	Molecular ion	Base peak	Other typical peaks I	II	III
Epichlorohydrin	0.22	92	57	27	31	49
Epoxibutane	0.20	72	42	39	41	57
Carbon tetrachloride	0.05	–	117	35	47	82
Chloroform	0.01	118	83	35	47	–
1.1.1-Trichloroethane	0.035	–	97	27	61	117
Diisobutylene (2.2.4-Trimethylpentene-1)	0.020	112	57	41	69	97
Ethylacetate	0.052	88	45	29	43	59
Pentanol-2	0.015	–	45	31	59	60
Butanol-2	0.051	74	45	31	59	73

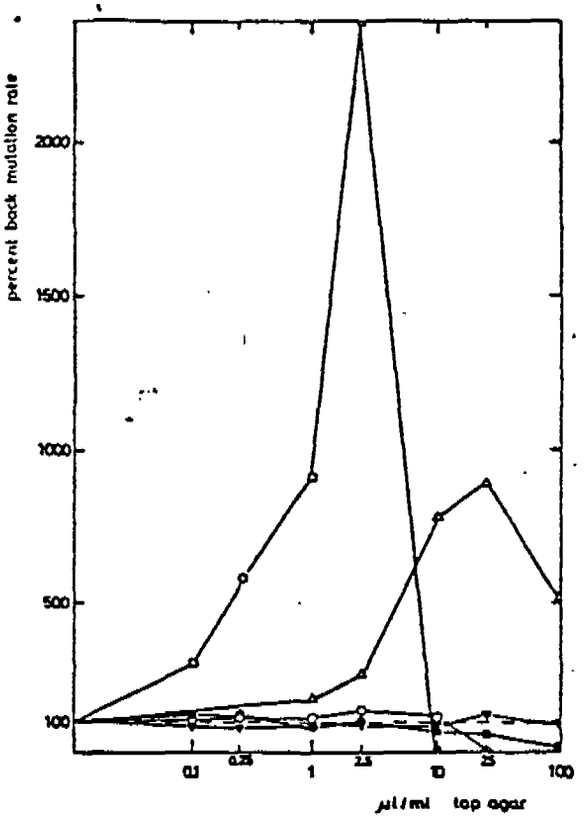

Fig. 14 Mutagenicity in the Ames in vitro system using *S. typhimurium* TA 100 (Ames et al., 1973) of trichloroethylene and contaminants identified in a technical sample. (□) Epichlorohydrin; (Δ) Epoxibutane; (▲) Diisobutylene; (○) Trichloroethylene. Open symbols: without, filled symbols: with addition of PCB (Aroclor 1254) induced rat liver microsomes. The bacteria were grown in nutrient broth, shaken for 12 h at 37° C, and 0.1 ml was then added to the molten top agar (2 ml), with and without 0.5 ml of "S-9-mix". This mix contained per ml: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP, 100 mM sodium phosphate (pH 7.4) and 0.3 ml of liver homogenates (S-9) (9000 x g supernatant) from male Wistar rats (of about 250 g each) which were induced by a single i.p. injection of a polychlorinated biphenyl (PCB) mixture (Aroclor 1254), diluted in corn oil to a concentration of 200 mg/ml. A dosage of 500 mg/kg was given to each rat 5 days before sacrifice. The solutions tested were added directly to the top agar. Triplicate petri plates containing Vogel-Bonner E medium were overlayed with this mixture and incubated at 37° C. After 48 h the revertant colonies were counted. Spontaneous back mutation rate 138 ± 8 (without microsomes) and 242 ± 10 (with microsomes) colonies per plate

Table 4 Tumors and tumor-bearing animals in mice after 18 months trichloroethylene inhalation (0; 100 and 500 ppm, 6h/day, 5 days/week). Number of tumor-bearing animals in brackets.

	males			females		
No. of autopsied animals	Contr. (30)	100 ppm (29)	500 ppm (30)	Contr. (29)	100 ppm (30)	500 ppm (28)
Benign tumors	8[a] (8)	7[b] (6)	4[c] (3)	7[d] (6)	1[e] (1)	4[f] (4)
Malignant tumors	14[g] (11)	12[h] (10)	9[i] (9)	14[j] (13)	25[k] (22)	23[l] (22)
number of which are malignant lymphomas	7	7	6	9	17	18
Tumors of undetermined class	1[m]	1[n]	0	4[o]	0	2[p]
Tumors (total)	23	20	13	25	26	29

a) 1 papillary bronchiolo-alveolar (alv.) adenoma, 1 hepatocellular adenoma, 1 myoma of the prostate, 1 adenoma of the adrenal cortex, 4 renal cystadenomas,

b) 3 papill. bronchiolo-alv. adenomas, 2 hepatocell. adenomas, 1 renal adenoma, 1 renal cystadenoma,

c) 1 papill. bronchiolo-alv. adenoma, 1 adenoma of the adrenal cortex, 1 renal cystadenoma, 1 cyst of the testicle,

d) 3 papill. bronchiolo-alv. adenomas, 3 ovarian adenomas, 1 adenoma of the adrenal cortex,

e) 1 ovarian adenoma,

f) 1 papill. bronchiolo-alv. adenoma, 3 ovarian adenomas,

g) 5 papill. bronchiolo-alv. carcinomas, 1 hepatocell. carcinoma, 1 adenocarcinoma of the kidney

h) 1 spindle cell sarcoma of the skin, 3 papill. bronchiolo-alv. carcinomas, 1 adenocarcinoma in the abdomen,

i) 1 polymorphic sarcoma of the skin, 1 bronchiogenic squamous cell carcinoma, 1 carcinoma of the testicle,

j) 1 sweat gland carcinoma, 1 papill. bronchiolo-alv. carcinoma, 1 ovarian adenocarcinoma, 1 adenocarcinoma of the uterus, 1 adenocarcinoma of the intestine,

k) 1 sweat gland carcinoma, 1 adenosquamous carcinoma of the skin, 3 papill. bronchiolo-alv. carcinomas, 1 adenocarcinoma in the abdomen, 2 ovarian adenocarcinomas.

l) 1 basal cell carcinoma, 2 ovarian adenocarcinomas, 1 follicular carcinoma of the thyroid gland, 1 leiomyosarcoma

m) 1 trabecular hepatoma,

n) 1 mesenchymal liver tumor,

o) 4 granulosa cell tumors of the ovary,

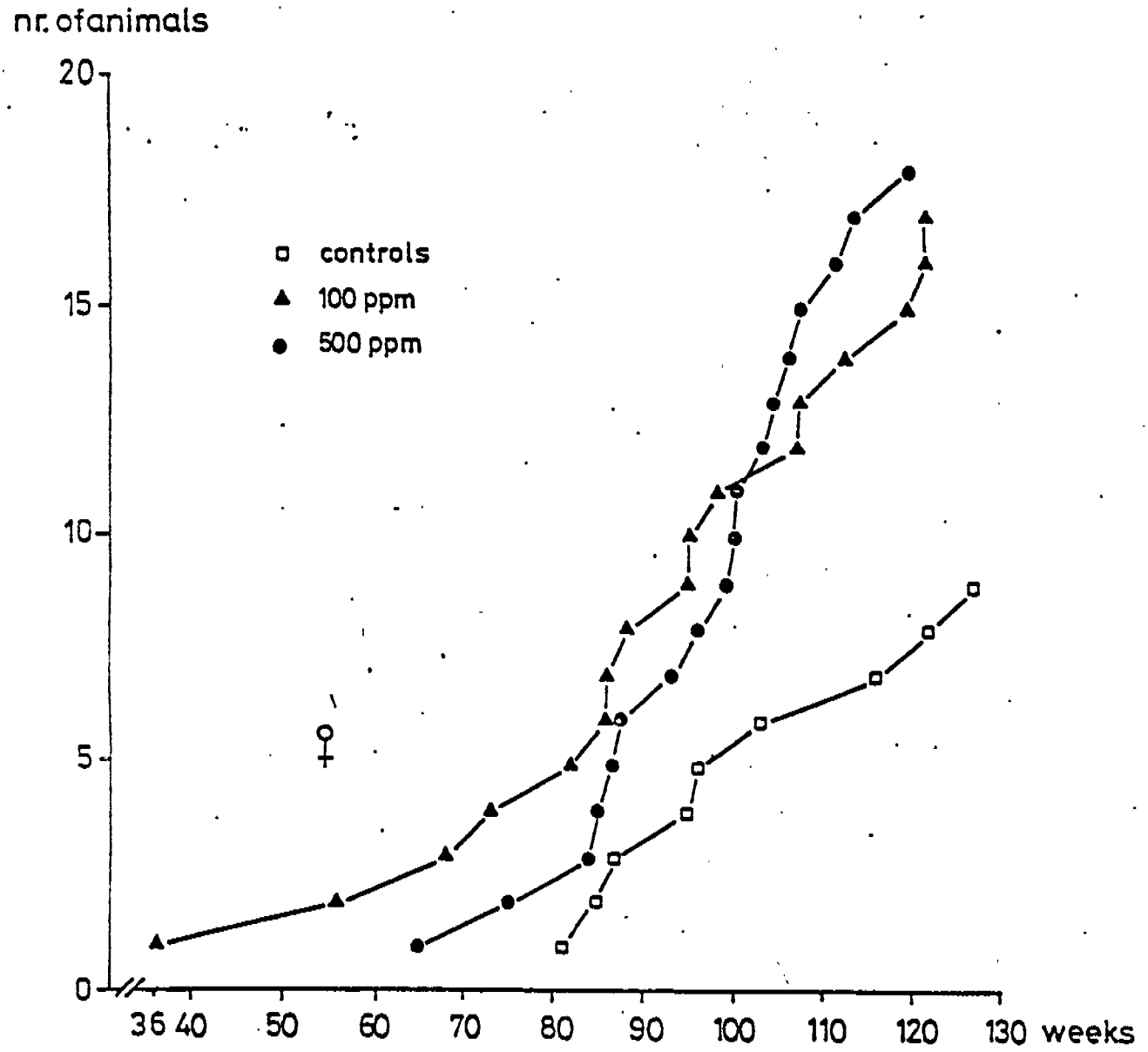

Fig. 15

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