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Information Section

ARTICLES OF GENERAL INTEREST

VINYL CHLORIDE—PART 3: MACROMOLECULAR BINDING

Chloroethylene oxide and chloroacetaldehyde are probably the principal metabolites of vinyl chloride (*Cited in F.C.T.* 1979, 17, 403). Evidence of the binding of these metabolites to cellular macromolecules, particularly the alkylation of the nucleic acids, may explain the mutagenicity of vinyl chloride (*ibid* 1979, 17, 542).

In vivo *studies*

The irreversible binding of VC metabolites to nucleic acid and protein has been demonstrated *in vivo* (Bolt *et al.* in IARC Scientific Publn no. 13, IARC, Lyon, 1976, p. 151). In rats exposed to atmospheres containing about 140 ppm [^{14}C]VC, roughly half of the radioactivity found in the liver microsomes immediately after the 5-hr exposure was irreversibly bound to protein, although the major proportion of labelled material in the liver cells was present in the cytosol. Immediately after exposure to 44 ppm [^{14}C]VC, from 10 to 40% of the total activity in the tissues was irreversibly bound, while at 48 hr the proportion of bound activity had increased to 70%, although the amount of irreversibly bound VC metabolites stayed the same. Radioactivity was also found in the liver nucleic acids. The peak incorporation of activity into DNA (0·23% total liver activity) occurred immediately after exposure, whereas the specific labelling of the RNA (0·8% of initial activity of the liver) was at a maximum after 24 hr and decreased more slowly. Bolt *et al.* (*loc. cit.*) calculated that as no more than 1% of the labelled VC would enter the one-carbon pool to be incorporated into protein and nucleic acid, this mechanism could not explain the total high level of binding observed in the study. It was possible that the one-carbon pool could account for the amounts of label found in the nucleic acid of the liver. However, the authors thought this unlikely since the speed by which this activity decreased was faster than normal DNA turnover and the rate of incorporation of label was very different in RNA and DNA derived from the same cellular nucleotide pool.

In further studies at the same institute (Laib & Bolt, *Arch. Tox.* 1978, 39, 235), liver RNA isolated from rats exposed to VC atmospheres was shown to contain labelled 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine, the alkylation products of adenine and cytosine by chloroethylene oxide and chloroacetaldehyde, as well as the physiological bases. The time-course of dealkylation was found to differ in the two bases. Ninety-two hours after exposure, the level of labelled ethenoadenosine was only one-fifth of its original value; ethenocytidine proved more resistant to repair and the levels of radioactivity associated with this component remained constant over the same period—possibly an indication of the relative importance of cytidine alkylation.

Watanabe *et al.* (*Toxic. appl. Pharmac.* 1978, 44, 571) exposed Sprague-Dawley rats to 1–5000 ppm ^{14}C-labelled VC for 6 hr. The amount of radioactivity bound to macromolecules in the liver did not increase in proportion to the increase in exposure concentrations. Covalent binding to hepatic macromolecules was related to the amount of VC metabolized. There was no detectable binding of ^{14}C to either DNA or RNA in the liver, in contrast to the results of Bolt *et al.* (*loc. cit.*). The authors thought this difference in results might be explained by the higher specific activity used by Bolt *et al.* They also offered the suggestion that the incorporation observed in the earlier work might have originated from the one-carbon pool, but this would not explain the alkylated bases isolated by Laib & Bolt (*loc. cit.*). Watanabe *et al.* (*loc. cit.*) concluded that covalent binding to hepatic nucleic acids occurred to only a limited degree under their experimental conditions but they pointed out that this did not exclude the possibility of other interactions which might result in the loss of the ability to control replication. Hepatic glutathione content was depressed at exposure concentrations above 100 ppm, and according to these authors reactive metabolites of VC may be detoxified by glutathione, suggesting that the carcinogenicity of VC may be related to a decreased ability to detoxify reactive metabolites.

The *in vivo* binding of VC metabolites to DNA has also been investigated in mice (Osterman-Golkar *et al. Biochem. biophys. Res. Commun.* 1977, 76, 259). BALB mice took up radioactivity about twice as fast as the CBA and ATL strains. Levels of *S*-(2-hydroxyethyl)cysteine, N^1- and N^3-hydroxyethylhistidine or N^7-hydroxyethylguanine were measured in the hydrolysates of proteins and nucleic acids taken from mice exposed to 98–302 ppm; these compounds were formed from the chemical (sodium borohydride) reduction of 2-oxoethyl groups introduced into the cell macromolecules as a result of the VC treatment. The histidine and guanine derivatives and small amounts of hydroxyethylcysteine were detected in hydrolysates of haemoglobin and liver DNA. The authors calculated that the absolute and relative amounts of alkylated products supported the hypothesis that the main reactive metabolite of VC is chloroethylene oxide. In the BALB mice, the two hydroxyethylhistidines could be detected in small amounts in the protein from the testes.

In vitro *studies*

The binding of the metabolites of VC to cellular macromolecules has also been studied by incubating rat-liver microsomes with NADPH, polyadenosine and [1,2-^{14}C]VC (Laib & Bolt, *Toxicology* 1977, 8, 185). The radioactivity identified in the enzymic hydrolysates of the polyadenosine was irreversibly attached to 1,N^6-ethenoadenosine. An analogous reaction occurred when polycytidylic acid was incu-

bated with VC and the microsomal system, labelled 3,N^4-ethenocytidine moieties being formed (Laib & Bolt 1978, *loc. cit.*).

The reaction of chloroacetaldehyde with calf-thymus DNA at pH 4·5 gave a modified DNA product (Green & Hathway, *Chemico-Biol. Interactions* 1978, 22, 211). Enzyme hydrolysis of the DNA produced a mixture of naturally occurring deoxyribonucleosides and chloroacetaldehyde reaction products, predominantly ethenodeoxycytidine and ethenodeoxyadenosine. The same products resulted from hydrolysis of liver DNA prepared from rats that had been exposed to 250 ppm VC in drinking-water for 2 yr.

According to the studies of Kappus *et al.* (*Toxic. appl. Pharmac.* 1976, 37, 461), the alkylation of protein and uptake of VC by rat-liver microsomes is dependent on concentration, incubation time, enzymatic activity, NADPH and oxygen, and is almost completely blocked by CO. These authors found that only about 1% of the VC taken up by the microsomes became irreversibly bound, a finding that contrasts with the corresponding figure of around 50% observed in the *in vivo* studies of Bolt *et al.* (*loc cit.*). Glutathione added to the microsomal incubation mixture decreased the level of irreversible protein binding. When trichloropropene oxide, an inhibitor of epoxide hydrase, was present, the irreversible protein binding increased two-fold, even though VC uptake by the microsomes was unaffected.

Other *in vitro* studies have produced evidence of chloroethylene oxide's participation in the metabolism of VC. The reaction product of chloroacetaldehyde or chloroethylene oxide with adenosine was tentatively characterized by Barbin *et al.* (*Biochem. biophys. Res. Commun.* 1975, 67, 596) as 3,β-ribofuranosylimidazo-(2,1-*i*)-purine (1,N^6-ethenoadenosine); a product with the same R_F value and elution characteristics on a Sephadex column was formed when VC was incubated with adenosine in the presence of a microsomal fraction. Barbin *et al.* (*loc. cit.*) passed a mixture of VC and air or oxygen into a medium containing both liver microsomes from a phenobarbitone-treated mouse and an NADPH-generating system. The volatile metabolite trapped by reaction with 4-(4-nitrobenzyl)pyridine (4-NBP) in ethylene glycol had a UV absorption spectrum identical to the product formed from the reaction of chloroethylene oxide (but not 2-chloroacetaldehyde) with 4-NBP.

The evidence from these studies strongly supports the view that chloroethylene oxide is the major reactive VC metabolite. While it is clear that metabolites of VC do bind to cellular macromolecules in rat and mouse liver, the extent of nucleic acid binding has not been clearly resolved.

[J. Hopkins—BIBRA]

BLACK MARKS FOR MBK

The industrial solvent methyl *n*-butyl ketone (MBK) has been implicated in cases of peripheral neuropathy among spray-painters and other workers (*Cited in F.C.T.* 1977, 15, 159) and has been shown to induce axonal degeneration of the central nervous system and polyneuropathy in experimental animals (*ibid* 1977, 15, 492).

The metabolic fate and disposition of MBK in the rat has been studied by DiVincenzo *et al.* (*Toxic. appl. Pharmac.* 1977, 41, 547) in male rats dosed by gavage with [1-^{14}C]MBK at 20 or 200 mg/kg. Absorption was rapid, and activity was eliminated in the breath and urine, mostly within 2 days. Unchanged MBK in the expired air amounted to 6·2% of the dose after 200 mg/kg and to 2·2% after 20 mg/kg. Total activity in the breath represented about 44% of either dose, with unchanged MBK and CO_2 as the only labelled compounds. Excretion of ^{14}C in the urine amounted to 35% after 20 mg/kg and to 40% after 200 mg/kg; faecal excretion of ^{14}C was less than 1·5% of the dose. About 15% of radioactivity remained in the carcass after 48 hr and 8% after 6 days. It was widely distributed throughout the tissues with highest concentrations in the blood and liver. The elimination time for MBK in the serum was about 6 hr, and the serum metabolites were 2-hexanol, 5-hydroxy-2-hexanone and 2,5-hexanedione. These three metabolites were also detected in the urine, together with 2,5-dimethylfuran, norleucine, γ-valerolactone and urea. 2-Hexanol was probably eliminated in the form of both the sulphate ester and the glucuronide.

These findings identify the principal metabolic pathways as reduction of the ketone group or oxidation at the α or ω-1 carbon, followed apparently by decarboxylation of the metabolites with an α-keto acid component, the latter stage being the probable source of most of the respiratory $^{14}CO_2$. The α-keto acid intermediates may also undergo transamination to amino acids. Pretreatment of rats with unlabelled MBK or with phenobarbital did not materially alter the metabolism of [1-^{14}C]MBK, but inhibition of the microsomal mixed-function oxidase system by pretreatment with SKF 525A increased $^{14}CO_2$ excretion from 37·6 to 49·6%, after an initial 4-hr decrease, and decreased urinary activity from 39·9 to 22·5%, indicating the involvement of this microsomal-enzyme system in the ω-1 oxidation of MBK.

DiVincenzo *et al.* (*ibid* 1978, 44, 593) continued their studies with an investigation of the respiratory uptake and percutaneous absorption of MBK in dogs and man. Male beagles exposed to 50 or 100 ppm MBK vapour for 6 hr had average breath concentrations of 16 and 35 ppm MBK, respectively, indicating an absorption rate of 65–68%. On cessation of exposure, the concentration of MBK in the breath fell rapidly and was below the level of detection after 3–5 hr. In male volunteers exposed to 10 or 50 ppm MBK for 7·5 hr or to 100 ppm MBK for 4 hr, mean

Title → K-17:1 -(000)
K-2:17 -
K-2:19 -

91: 103405u Mutagenicity of vinyl chloride, vinylidene chloride and chloroprene in V79 Chinese hamster cells. Drevon, C.; Kuroki, T. (Unit Chem. Carcinog., Int. Agency Res. Cancer, 69372 Lyon, 2 Fr.). *Mutat. Res.* 1979, 67(2), 173–82 (Eng). The mutagenicity of *vinyl chloride* [75–01–4], *vinylidene chloride* [75–35–4], and *chloroprene* [126–99–8] was tested in V79 Chinese hamster cells in the presence of a 15,000 × *g* liver supernatant from phenobarbitone–pretreated rats and mice. Mutations in terms of 8–azaguanine and ouabain resistance were induced in a dose–related fashion by exposure to vapor of vinyl chloride in the presence of liver supernatant from phenobarbitone–pretreated rats. Vapors of vinylidene chloride and chloroprene induced a dose–related toxicity in the presence of liver supernatant from phenobarbitone–pretreated rats, but these 2 compds. were not mutagenic in V79 Chinese hamster cells under the present assay conditions. The results are discussed with regard to the metabolic activation of the compds. and to the correlation with their carcinogenicity in man and exptl. animals.

PN

91: 78393d **Comparative evaluation of the frequency of chromosomal aberrations and the sister-chromatid exchange (SCE) numbers in peripheral lymphocytes of workers occupationally exposed to vinyl chloride monomer.** Kucerova, M.; Polivkova, Z.; Batora, J. (Pediatr. Dep., Postgrad. Med. Inst., Prague, Czech.). *Mutat. Res.* 1979, 67(1), 97-100 (Eng). Comparison of data on 9 workers exposed to *vinyl chloride* (I) [75-01-4] indicates that routine chromosomal aberrations and SCE nos. are equally suitable for testing of high-dose I mutagenicity in vivo.

R&S 133357

PV

12-178-(155)

90: 98232b **Embryotoxic and teratogenic effects of vinyl chloride.** Mirkova, E.; Mihailova, A.; Nosko, M. (Med. Akad., Sofia, Bulg.). *Khig. Zdraveopaz.* 1978, 21(5), 440-3 (Bulg). Continuous inhalation of 6.15 mg *vinyl chloride* [75-01-4]/m^3 during 21 days of pregnancy increased ≥8 fold early postimplantation resorption of rat embryos, without affecting the late postimplantation mortality. The frequency of hematomas increased 8 fold, ossification of the skeleton was disturbed, and external and internal malformations occurred. Internal hydrocephaly occurred in 54.4% of the fetuses and encephalocoele in 2.53%. The wt. of fetuses was decreased. In 1-mo-old pups the hexobarbital sleep was prolonged, and the impairment of detoxifying function of the liver persisted in 2-mo-old animals. The level of cholic acid and the enzyme activity in the bile were decreased, indicating development of an early toxic hepatopathy. The rate of bile secretion was decreased.

CA Selects
Issue 9, 1979

90: 141993w **Nature and frequency of sleep disorders in persons occupationally exposed to the effects of vinylchloride.** Gnesina, E. A.; Antonyuzhenko, V. A.; Mashikhin, E. N.; Rostovtseva, G. G. (Inst. Gig. Tr. Profzabol., Gorkiy, USSR). *Gig. Tr. Prof. Zabol.* 1978, (11), 16-19 (Russ). Examns. were given to 158 workers exposed to *vinyl chloride* [75-01-4]. The neurol. status and the subjective evaluation of sleep were studied. The dyssomnia syndrome was obsd. in 70% of the participants. M. Kowalski

K-1711-(100) P

92: 46720n **Monitoring for chromosomal damage in exposed industrial populations.** Kilian, D. Jack; Picciano, Dante J. (Occup. Health and Med. Res., Dow Chem. U.S.A., Freeport, TX USA). *Genet. Damage Man Caused Environ. Agents, [Proc. Conf.]* 1977 (Pub. 1979), 101-115 (Eng). Edited by Berg, Kaare. Academic: New York, N. Y. No cytogenetic differences of significance were found between the group of *vinyl chloride* (I) [75-01-4]-exposed workers and the control group. Ninety-five percent of the workers exposed to >5 ppm I belonged to the group with 0-5% aberrations and 5% belonged to the group with >5% aberrations, as compared to 94% and 6% for the control group.

92: 46724s **A follow-up study of PVC workers two years after exposure. Preliminary results using sister chromatid exchange frequency as an assay of genetic damage.** Hansteen, Inger Lise (Lab. Genet., Telemark Cent. Hosp., Porsgrunn, Norway). *Genet. Damage Man Caused Environ. Agents, [Proc. Conf.]* 1977 (Pub. 1979), 279-85 (Eng). Edited by Berg, Kaare. Academic: New York, N. Y. There is no significant difference in the frequency of sister chromatid exchanges (SCEs) between *PVC* [9002-86-2] workers and their matched controls 2 yr after exposure to 1 ppm *vinyl chloride* [75-01-4]. The mean SCEs of exposed workers is 7.6 (range 4.7-10.5), as compared to 7.5 (range 5.1-11.1) for the control.

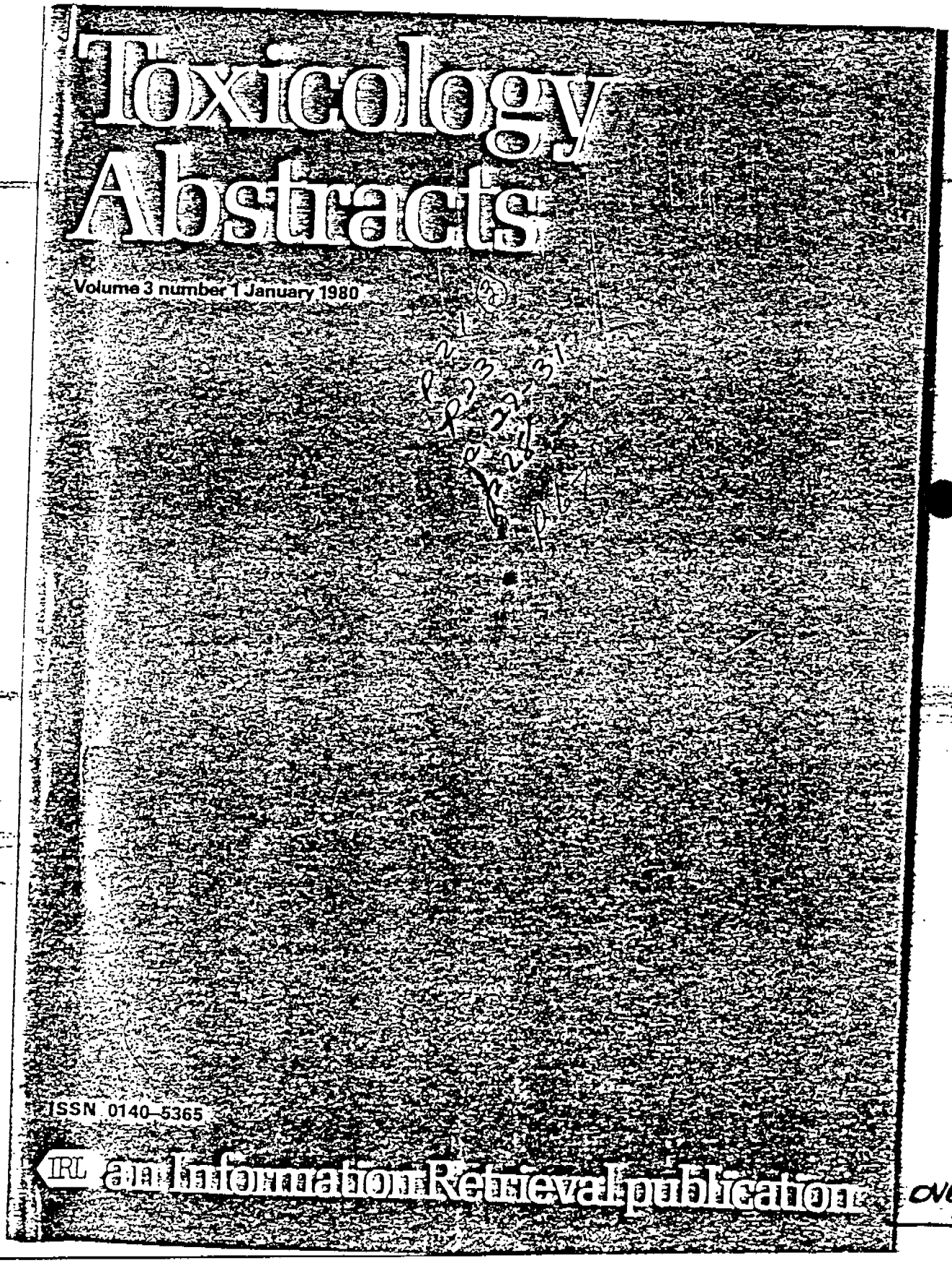
R&S 133359

K-1711 - (100)

PV

92: 152259d **Pathological skin lesions in workers with long-time exposure to vinyl chloride.** Czernielewski, Antoni; Kiec-Swierczynska, Marta; Gluszcz, Maria; Wozniak, Leszek (Klin. Dermatol., 94-017 Lodz, Pol.). *Przegl. Dermatol.* 1979, 66(5), 463-9 (Pol). Extensive investigations have shown that histopathol. examn. of skin samples collected from the extremities of workers subjected to long-time exposure to *vinyl chloride* [75-01-4] represents the basic method by which the effects of such exposure can be detd. Histol. skin changes seem to be the earliest symptoms of chronic poisoning caused by the toxicity of vinyl chloride, and they can be recognized before any clin. symptoms become noticeable. E. A. Ackermann

215-X3 **Vinyl chloride and trichloroethylene: comparison of alkylating effects of metabolites and induction of preneoplastic enzyme deficiencies in rat liver.** Laib,R.J.; Stockle,G.; *Bolt,H.M.; Kunz,W. (Inst. Toxikol.,Univ. Tubingen, Wilhelmstr. 74, D-7400 Tubingen, GFR) *J. Cancer Res. Clin. Oncol., 94(2), 139-147 (1979)* En:en.
[1,2-^{14}C] Vinyl chloride and [1,2-^{14}C] trichloroethylene were incubated with rat liver microsomes, NADPH and RNA (from yeast). Whereas trichloroethylene metabolites were irreversibly bound to proteins in microsomal incubations to a higher extent than vinyl chloride metabolites, irreversible binding to RNA was lower for trichloroethylene metabolites. Hydrolysis of the RNA which was reisolated from microsomal incubations with ^{14}C-vinyl chloride or ^{14}C-trichloroethylene and separation of the nucleosides showed different alkylation products arising from vinyl chloride and from trichloroethylene, characteristic for vinyl chloride being formation of 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine. The different reactivities of metabolites of vinyl chloride and of trichloroethylene prompted a comparison of the oncogenic effects of both compounds against the rat liver cell. Newborn rats were exposed for 10 wk to 2000 ppm vinyl chloride or trichloroethylene (8 h/day; 5 days/wk). After this period livers of the animals were stained for nucleoside-5-triphosphatase. Whereas the vinyl chloride exposed rats showed focal hepatocellular deficiencies in this enzyme, which are supposed to represent an early sign of malignancy, no such changes were induced by trichloroethylene exposure. The data therefore suggest differences between the hepatocarcinogenic activity of vinyl chloride and possible effects of trichloroethylene on the liver.



R&S 133362

OVER →

K-1711-(100)P V
K-2504R ✓

92: 16610j Acute hepatoxicity of vinyl chloride and ethylene: modification by trichloropropene oxide, diethylmaleate, and cysteine. Conolly, R. B.; Jaeger, R. J. (Dep. Physiol., Harvard Sch. Public Health, Boston, MA 02115 USA). *Toxicol. Appl. Pharmacol.* 1979, 50(3), 523–31 (Eng). The present study used several chem. treatments to investigate the mechanisms of acute *vinyl chloride* (VC) [75-01-4] and *ethylene* [74-85-1] toxicity in polychlorinated biphenyl (PCB)–pretreated rats. *Trichloropropene oxide* (TCPO) [3083-23-6] depleted hepatic *glutathione* (GSH) [70-18-8] and inhibited hepatic *epoxide hydrase* (EH) [9048-63-9]. *Diethylmaleate* (DEM) [141-05-9] also depleted hepatic GSH. *Cysteine* [52-90-4] was a rate–limiting precursor in hepatic GSH synthesis. Effects of these various treatments on VC and ethylene toxicity were compared with their influence on hepatic GSH concns. Exposure to VC and ethylene were by inhalation at 1000–50,000 ppm. TCPO increased VC toxicity in fasted, but not in fed, rats. Ethylene toxicity was not affected by TCPO. These actions of TCPO were not attributable to changes in hepatic GSH. DEM lowered GSH during exposure, but did not increase the hepatotoxicity of either VC or ethylene. This suggests either that the hepatic GSH concn. is not an important determinant of the acute response to VC and ethylene or that DEM has important effects in addn. to hepatic GSH depletion. Cysteine protected significantly, although incompletely, against acute VC hepatotoxicity. There was no effect of cysteine on the toxicity of ethylene. Thus, in PCB-treated rats, hepatic GSH and EH influence the acute hepatotoxicity of VC, but not that of ethylene.

R&S 133363