R&S 107896

BIO-MEDICAL RESEARCH
DOCUMENT DESCRIPTION FORM

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VINYL CHLORIDE—PART 1: METABOLISM

The period of phrenetic activity—or at least sustained debate—on the status of vinyl chloride (VC) seems to have passed. Since technological feasibility eventually formed the basis of the first generation of VC legislation, the authorities are now biding their time, pending the next generation of biological data, which will allow an assessment of the hazards associated with the currently permitted levels. We last reviewed VC early in 1976 (BIBRA Bull. 1976, *15*, 6 & 72). In the intervening period the biochemists and microbiologists have demonstrated particular interest in this monomer.

Dose-related metabolism

A preliminary study by Dow Chemical USA on the fate of unlabelled VC in the rat indicated that its metabolism was dependent on the level of exposure (Hefner *et al.* Ann. N.Y. Acad. Sci. 1975, *246*, 135). In the range 50–105 ppm the half-life was 86 min, whereas in the range 220–1167 ppm the half-life was 261 min. Following up this work, the same group investigated the metabolism of ^{14}C-labelled VC in the rat (Watanabe *et al.* Toxic. appl. Pharmac. 1976, *37*, 49). Rats exposed to 10 ppm VC for 6 hr eliminated 68% of the absorbed radioactivity in their urine and 2% (as VC) in the expired air over 72 hr; 6-hr exposures to 1000 ppm resulted in 56% of the dose appearing in the urine with 12% in the expired air as VC. The pulmonary excretion of the VC followed first-order kinetics with similar half-lives at both treatment levels: 20·4 min at 10 ppm and 22·4 min at 1000 ppm. Elimination of ^{14}C in the urine was more complex, being described by a two-exponential equation, but the rates of the initial phase were comparable; at 10 ppm the half-life was 4·6 hr while at 1000 ppm it was 4·1 hr. After 72 hr, 14–15% of the administered radioactivity of both the high and low doses was recovered from the carcases, indicating, when correction is made for the amount of VC metabolized, that there is increased storage of radioactivity at higher doses.

The metabolism of VC administered by other routes appears also to be dose-dependent. Indeed an even greater disparity between the metabolism of high and low doses seems to follow oral administration. In another study by Watanabe *et al.* (*ibid* 1976, *36*, 339) from 59 to 68% of an oral dose of 0·05 or 1 mg VC/kg was converted to non-volatile urinary metabolites, with 9–13% appearing as expired CO_2 and only 1–2% being excreted by the lungs as unchanged VC. By contrast, 11% of the highest dose of 100 mg/kg was excreted in the urine, and 67% was excreted as VC (and 3% as CO_2) in the expired air. The pulmonary excretion at 100 mg/kg was biphasic, with half-lives of 14·4 and 40·8 min, whereas at 0·05 and 1 mg/kg it was monophasic with a half-life of about 55 min.

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Similar findings were reported by the ICI workers, Green & Hathway (Chemico-Biol. Interactions 1975, *11*, 545), low intragastric doses of VC being eliminated principally in the urine whilst higher doses were mainly excreted unchanged via the lungs. About three-quarters of an intubated dose of 250 µg [^{14}C]VC/kg was converted to non-volatile urinary metabolites and 12-15% was excreted in the expired air as CO_2 together with a few per cent as unchanged monomer. By contrast, at a dose of 450 mg/kg pulmonary excretion predominated, with 90% of the radioactivity being associated with unchanged VC and less than 1% with CO_2. The pulmonary elimination of monomer, shown to be proportional to the log of reciprocal dose, was completed within 5 hr, whereas both the excretion of CO_2 via the lungs and the elimination of non-volatile metabolites in the urine continued over 72 hr. Similar excretion kinetics were observed in rats that had been pretreated for 60 days with unlabelled VC at doses up to 300 mg/kg/day (Green & Hathway, *loc.cit.*).

Withey (J. Toxicol. envir. Hlth 1976, *1*, 381) was unconvinced that intubation was an acceptable method of administering VC in a metabolic investigation, for he observed wide inter-animal variability in his studies in which rats with a cannulated jugular vein were intubated with aqueous and oil solutions of VC. The uptake of VC was rapid, peak concentrations being achieved within 10 min, with blood levels varying from 6 to over 40 µg VC/ml.

The metabolism of VC given by iv and ip injection was also studied at ICI (Green & Hathway, *loc.cit.*). Almost all of a small (250 µg/kg) iv dose was found in the expired air as VC. About 43% of the same dose given by ip injection was excreted in the expired air as VC, another 43% being converted to urinary metabolites and 11% to CO_2. An ip dose of 450 mg VC/kg was almost totally excreted (96%) as VC in the expired air, with only 2·6% being metabolized to non-volatile species excreted in the urine.

Identity of metabolites

Identification of the urinary metabolites was attempted in both the Dow and ICI studies. In the oral and inhalation studies of Watanabe *et al.* (*loc.cit.*), most of the radioactivity excreted in the urine of rats was associated with three compounds, the proportions of which were unaffected by the dose or by the route by which the VC was administered. *N*-Acetyl-*S*-(2-hydroxyethyl)cysteine accounted for 29-40% and thiodiglycollic acid for 18-26% of the radioactivity detected in the urine, with the unidentified third compound being present at levels of from 30 to 39%. In the oral experiments, the three metabolites represented from 91 to 95% of the total radioactivity excreted in the urine, whilst in the inhalation experiments the corresponding figure was 96-97%.

The first report by Green & Hathway (*loc.cit.*) of their investigations of the metabolism of orally administered [^{14}C]VC (150 mg/kg) in the rat, noted that the three major urinary metabolites were thiodiglycollic acid (47% of the urinary radioactivity), *S*-(2-chloroethyl)cysteine (23%) and *N*-acetyl-*S*-(2-chloroethyl)cysteine (23%). The remaining radioactivity in the urine was associated with urea (6%), glutamic acid (0·5%), chloroacetic acid (0·5%), methionine (trace) and serine (trace). Watanabe *et al.* (Toxic. appl. Pharmac. 1976, *36*, 339) were critical of these results, suggesting that the chloroethyl compounds were artefacts formed

by the reaction of the hydroxy analogues with the hydrochloric acid gas used in the derivatization procedure. This criticism was accepted by Green & Hathway who reported in their most recent paper (Chemico-Biol. Interactions 1977, *17*, 137) that the major urinary metabolites of VC in the rat were *N*-acetyl-*S*-(2-hydroxyethyl)cysteine, the related *N*-acetyl-*S*-vinylcysteine and thiodiglycollic acid.

Further elucidation of VC's metabolic pathway(s) was attempted by Green & Hathway (1977, *loc.cit.*), who measured the yields of thiodiglycollic acid in the urine of rats given the various possible metabolites intragastrically at high doses. Thiodiglycollic acid in 0·5% theoretical yield was isolated in the urine of rats dosed with *S*-(2-hydroxyethyl) cysteine, a result said to be highly significant in view of the cysteine derivative's instability in even mild reaction conditions. Furthermore, as high yields of the acid were found in those animals dosed separately with chloroacetaldehyde, chloroacetic acid and *S*-(carboxymethyl)cysteine, it was suggested that all these compounds might lie on a common metabolic pathway linking VC with thiodiglycollic acid. Nevertheless, the fact that only small amounts of chloroacetic acid (<0·1%) were detected in the body fluids of animals treated with the high doses of VC, backed by evidence from the metabolism of chloroacetaldehyde (and vinylidene chloride), suggested that chloroacetic acid is not a major VC metabolite. Green & Hathway (1977, *loc.cit.*), believed that in rats, chloroethylene oxide was formed from VC and might then be transformed spontaneously into chloroacetaldehyde. The chloroacetaldehyde or chloroethylene oxide reacts principally with glutathione, catalysed by glutathione-*S*-epoxide transferase, to form *S*-(2-acetal)cysteine with subsequent formation of *S*-(2-hydroxyethyl)cysteine and its *N*-acetyl derivative, *S*-(carboxymethyl)-cysteine and thiodiglycollic acid.

It was the view of Hefner and his colleagues (*loc.cit.*) in 1975 that VC was metabolized at low exposures, below 100 ppm, to 2-chloroethanol, chloroacetaldehyde and monochloroacetic acid via the alcohol dehydrogenase pathway. Only small amounts of the chloroacetic acid were formed at these low doses, due to the rapid reaction of chloroacetaldehyde with the sulphydryl groups of glutathione and cysteine. It was thought that at higher exposures (200-1000 ppm) direct epoxidation by microsomal oxidases occurred, with rearrangement of the resulting chloroethylene oxide to chloroacetaldehyde. However, the more recent study of Bolt *et al.* (Arch. Tox. 1976, *35*, 153) showed that in rats exposed to the monomer at an initial atmospheric concentration of 50 ppm in a closed system, the uptake of VC was completely blocked initially by 3-bromophenyl-4(5)-imidazole or 6-nitro-1,2,3-benzothiadiazole, both inhibitors of cytochrome *P*-450-dependent metabolism and was increased by DDT pretreatment, suggesting that the microsomal oxidases may be operative even at low doses. Weaker inhibitors of this enzyme system, such as SKF-525A or 5,6-dimethyl-1,2,3-benzothiadiazole, produced a measurable but lower order of inhibition. In the original Dow work (Hefner *et al. loc.cit.*), SKF-525A had no effect on the metabolism of low doses of VC (65 ppm) but did slightly decrease metabolism at VC exposures of the order of 1000 ppm.

Cytochrome P-450 involvement

Liver microsomal fractions have been shown to interact with VC *in vitro* (Salmon, Cancer Lett. 1976, *2*, 109). The addition of VC to rat-liver microsomes and NADPH produced a Type 1 spectral shift, similar

to that seen for phenobarbital, thus indicating direct involvement of a cytochrome *P*-450 species. Other *in vitro* studies have also demonstrated that the oxidation of VC to non-volatile products was dependent both on microsomal enzymes and on NADPH (Kappus *et al.* Toxic. appl. Pharmac. 1976, *37*, 461).

It has been suggested that VC metabolites can destroy cytochrome *P*-450. Ivanetich *et al.* (Biochem. biophys. Res. Commun. 1977, *74*, 1411), for example, showed not only that the binding and metabolism of VC *in vitro* was fully inhibited by SKF-525A, indicating the involvement of the cytochrome *P*-450 system, but that the VC metabolites reduced the level of both cytochrome *P*-450 (reduced to *P*-420) and microsomal haem. The levels of cytochrome b_5 or NADPH-cytochrome *c* reductase were unaffected by exposure to the monomer. Decreases in the level of cytochrome *P*-450 and in the total activity of hepatic microsomal enzymes were observed in rats 24 hr after they had been exposed for 6 hr to a 5% VC atmosphere (Reynolds *et al.* Res. Commun. chem. Path. Pharmac. 1975, *12*, 685). The selective enzymatic deactivation was consistent with a cytochrome *P*-450-centred activation of VC to reactive electrophiles. The destruction of cytochrome *P*-450 was found to be inhibited by glutathione in the study of Ivanetich *et al.* (*loc.cit.*), whereas Guengerich & Strickland (Molec. Pharmacol. 1977, *13*, 993) reported that neither added reduced glutathione nor cysteine offered any protection against the VC-mediated destruction.

The loss of cytochrome *P*-450 activity was attributed by Guengerich & Strickland (*loc.cit.*) to the destruction of haem, and not to lipid peroxidation or the binding of electrophiles to free sulphydryl groups. Since the destruction of *P*-450 required all the components necessary for mixed-function oxidation, it was said that the oxidative metabolism of VC by cytochrome *P*-450 was necessary for the observed destruction. The investigators did not consider, however, that the two commonly proposed VC-metabolites, the epoxide and chloroacetaldehyde, were the agents responsible for the cytochrome *P*-450 loss, as neither compound proved particularly effective at destroying either free or *P*-450-bound haem *in vitro*. Consequently it was postulated that different mechanisms underlie VC's mutagenic activity and its ability to destroy *P*-450. The latter is localized at the activating enzyme, consistent with a highly reactive species, whereas mutagenesis seems to require a metabolite stable enough to be transported from its site of activation to interact with nucleic acids or associated macromolecules.

Comment

The view that the microsomal mixed-function oxidases play an active and possibly the major role in the metabolism of VC monomer has received support from the demonstration that a microsomal-oxidase pathway operates at low VC exposures. The results of *in vitro* studies have also indicated that VC is metabolized by enzymes of the liver microsomes, in particular by a cytochrome *P*-450 species, to give initially chloroethylene oxide and 2-chloroacetaldehyde. Further investigation into VC-mediated destruction of cytochrome *P*-450 is required, but it would seem that here, too, a cytochrome enzyme is involved in the formation of the active metabolite(s).

The metabolic studies conducted by Dow and ICI have confirmed the dose-dependence of the fate of VC in the rat. It would seem, on the evidence now available, that in the rat there is effectively a single saturable metabolic pathway for VC. As the dose of VC increases, an

increased proportion escapes metabolism and is eliminated unchanged in the expired air. The fact that the major urinary metabolites—probably derived from the conjugation of chloroethylene oxide or 2-chloroacetaldehyde with glutathione—appear to be unaffected both qualitatively and quantitatively by dose tends to oppose the hypothesis of alternative pathways varying in importance in relation to dose. If there are a number of important metabolic pathways, then they appear to be leading to the same end products, an unlikely, although not impossible, occurrence.

[J. Hopkins]

GETTING RID OF PENTACHLOROPHENOL

As a bactericide, fungicide and herbicide, pentachlorophenol (PCP) finds use in a wide range of applications; in particular, it has become an important wood preservative. Its potential significance as a major environmental contaminant must therefore be recognized.

PCP appears to be readily absorbed through the skin and has been held responsible for aplastic anaemia, muscular paralysis and sensory loss in factory workers who failed to use adequate protective clothing and who worked in poorly ventilated areas (BIBRA Bull. 1965, *4*, 314). The toxicity of PCP to rats was shown to be greater when the compound was inhaled than after oral or subcutaneous administration (*ibid* 1977, *16*, 204). The question of PCP toxicity is complicated, however, by the fact that technical-grade PCP is frequently contaminated with varying amounts of toxic by-products, including other chlorinated phenols and, in some cases, chlorodibenzo-*p*-dioxins and chlorodibenzofurans. Thus Kimbrough & Linder (Toxic. appl. Pharmac. 1978, *46*, 151) found that whereas technical PCP fed for 8 months at a level of 500 or 100 ppm in the diet caused pronounced morphological changes in the livers of rats and some change was noted even with 20 ppm, no change was detected in rats fed 20 or 100 ppm purified PCP and only slight changes were apparent in those given 500 ppm in the diet.

Nevertheless, PCP itself has been shown to be an extremely efficient uncoupler of oxidative phosphorylation in mitochondria (Weinbach, J. biol. Chem. 1954, *210*, 545; Weinbach & Garbus, Nature, Lond. 1969, *221*, 1016) and to be capable of disturbing microsomal detoxication mechanisms in the liver (Arrhenius *et al*. Chemico-Biol. Interactions 1977, *18*, 35). By gas-chromatographic analysis of the sub-cellular fractions of the livers of rats treated orally with 0·15 mmol (*c*. 40 mg) PCP/kg, the latter group demonstrated that, compared with the cytosol, the mitochondria contained markedly lower concentrations of PCP and the microsomes showed a high accumulation (*idem*, *ibid* 1977, *18*, 23), suggesting that the effects of PCP on the detoxication functions of the endoplasmic reticulum are at least as relevant physiologically as the compound's capacity for mitochondrial uncoupling.

The fate of a single oral dose (10 or 100 mg/kg) of ^{14}C-labelled pure PCP was studied in rats for 9 days, with a final examination of tissues for residual activity (Braun *et al*. Toxic. appl. Pharmac. 1977, *41*, 395). The primary route of excretion of PCP was through the kidneys, with most of the remaining fraction of the dose appearing in the