

GENERAL DISCUSSION

The experimental results from the studies of the distribution and elimination of the investigated organic solvents have been discussed in detail chapterwise in relation to what was previously known about their toxic effects and fate in the living organism. Some additional comments and remarks regarding the general features of the distribution and elimination of the solvents selected for study will be given below.

LOW-TEMPERATURE AUTORADIOGRAPHY

The obvious and pronounced differences in the distribution patterns of the organic solvents registered by low-temperature autoradiography as compared to conventional autoradiography of dried whole-body sections demonstrate the importance and difficulties in selection of the proper experimental techniques for studies of volatile substances. The registration of the organic solvents themselves was thus possible only by working at a very low temperature. As expected, the high fat solubility of the solvents caused their uptake in the fat depots of the body and in the lipid-rich nervous tissues. The retention time of the solvents in these tissues was found to be dependent on their fat solubility, which among the aromatic hydrocarbon solvents increases with the number and length of the ring

alkyl substituents, i.e. in the order benzene, toluene, xylene, styrene and, among the chlorinated aliphatic hydrocarbon solvents with the degree of chlorination, i.e. in the order methylene chloride, chloroform, trichloroethylene, carbon tetrachloride. The solvents were always more rapidly cleared from nervous tissues than from fatty tissues, which is very probably due to the larger blood supply and perfusion of nervous than of fatty tissues. However, a comparison between the aromatic and the chlorinated aliphatic solvents gives at hand that the lipid solubility, commonly expressed as an oil/gas, fat/gas or oil/blood partition coefficient, may not be the only factor determining the retention time in nervous and fatty tissues, since unexpectedly long retention times in relation to their fat solubilities were noted for the chlorinated solvents. Thus, carbon tetrachloride, reported to have approximately the same or even a lower fat solubility than benzene (Appendix 2), was registered by low-temperature autoradiography in a large amount in fatty tissues still 8 hours and in nervous tissues still 4 hours after inhalation, whereas benzene was cleared from these tissues in 2 hours and 30 minutes, respectively. The autoradiographic results could thus indicate that the anesthetic potency of an organic solvent cannot be predicted on the basis of its lipid solubility only and that additional mechanisms of anesthesia and nervous system effects may exist such as the interaction of organic solvents with specific nervous tissue receptors. This may be the rule for the chlorinated solvents and the basic reason for their efficiency and use as clinical anesthetic agents, but it was clearly demonstrated in the case of chloroform, which showed a specific

localization to the cerebellar cortex, perhaps associated with reported chloroform induced cerebellar syndromes including ataxia, tremor and slurred speech (84, 176), meninges and spinal nerves at all survival times up to 48 hours after inhalation, making chloroform an outstanding and remarkable exception to the rule stating an exclusively physico-chemical relationship between the fat solubility of a substance and its localization and retention in nervous tissues.

As pointed out repeatedly in the discussions in the individual chapters in this monograph, no non-volatile radioactivity was ever registered in nervous tissues, which disagrees with the recently postulated theory (366) that short-lived reactive intermediates of organic solvents would be involved in the development of encephalopathy and long-lived reactive intermediates in the development of encephalopathy as well as neuropathy, both diseases which have been recognized as results of chronic exposure to organic solvents. The autoradiographic results would rather support a role of the solvents themselves in causing chronic neurotoxic effects. The stability of solvent biotransformation products and intermediates is largely unknown, but, generally, their polarity would make their passage into the nervous system difficult and their reactivity would enable them to travel only short distances from their sites of formation. The recent finding that the brain lacks cytochrome P450 activity (466) makes it unlikely that solvent biotransformation would take place *in situ* in nervous tissues.

AUTORADIOGRAPHY OF DRIED, EVAPORATED SECTIONS AND OF DRIED, EVAPORATED AND EXTRACTED SECTIONS

A common feature of the distribution patterns of the metabolites of the solvents selected for study was their accumulation in the liver and kidney, two organs known to be particularly affected at exposure to solvents. In the case of toluene and xylene all metabolites, including those registered in the liver and kidney, were completely extractable. Apart from their recognized effects on the nervous system at acute (51, 65, 137, 146, 300, 434) and, in the case of toluene, chronic (46, 159, 228, 365) exposure, toluene and xylene are believed to be two relatively harmless solvents with low hepato-renal toxicity (51, 52, 65, 103, 124, 198, 343, 434). Firmly bound benzene metabolites were registered both in the kidney and in the liver, which points out benzene as a possible hepato-renal toxic agent, a property which has been investigated and discussed (203, 204, 205, 206, 207, 210, 213, 449, 473) but attracted little attention compared to its effects on the blood-forming tissues. Firmly bound metabolites were registered not only in the liver after administration of styrene and trichloroethylene, in accordance with previously reported results showing a covalent binding of radioactivity from ^{14}C -styrene oxide, a presumed styrene metabolite, to rat liver macromolecules *in vitro* and *in vivo* (264) and from ^{14}C -trichloroethylene to rat and mouse liver proteins *in vitro* and *in vivo* (15, 44, 451, 459), but also in the bronchi and the kidney, which may indicate additional sites of action of metabolites formed from styrene and trichloroethylene. Firmly bound metabolites were registered

in the liver, kidney and bronchi also after inhalation of methylene chloride, chloroform and carbon tetrachloride. After inhalation of chloroform and carbon tetrachloride, both recognized as highly hepatotoxic agents producing centrilobular liver necrosis (38, 100, 182, 211, 238, 298, 401, 473), non-volatile firmly bound metabolites showed a characteristic, presumably centrilobular, liver distribution pattern, whereas this was not noted after inhalation of methylene chloride, which is considered a solvent of low hepatotoxicity (99, 103, 143, 222, 316, 345). Metabolites of chloroform, a potent nephrotoxin in mice (119, 193, 222, 317), were registered in a remarkably high concentration in the kidney cortex.

In addition to the general uptake of solvent metabolites in the liver and kidney, metabolites were registered interstitially in the testicle after inhalation of benzene, known to increase testicular weight and cause histochemical changes in the testes of rats (189, 475), chloroform, known to cause increased testicular weight in chronically exposed animals (444) and carbon tetrachloride, reported to cause testicular atrophy in experimental animals (4). Only in the case of carbon tetrachloride were the metabolites firmly bound. Firmly bound carbon tetrachloride metabolites were also registered in a remarkably high concentration in the gastro-intestinal mucosa, which may indicate a direct action of carbon tetrachloride also on other parts of the gastro-intestinal tract than the liver, in contrast to the previously suggested indirect, CNS mediated role of carbon tetrachloride in the production of gastro-intestinal disorders in chronically exposed workers (430).

Non-volatile, firmly bound radioactivity was also registered in a number of tissues with a rapid cell turnover or a high rate of protein synthesis after administration of several of the radiolabeled solvents due to the normal metabolic incorporation of radioactive one-carbon fragments formed during their biotransformation into various tissue macromolecules. This observation merits attention in several ways. Firstly, since radiolabeled substances are used in studies of the covalent binding of foreign compounds to tissue macromolecules, radioactivity found to be covalently bound must not uncritically be accepted as a result of the biotransformation of the compound to reactive intermediates or metabolites. Secondly, the problem of distinction between the normal incorporation of biotransformation products into tissue macromolecules and their covalent binding to various tissue components may be solved if there is a choice of label position in the molecule. A good example may be found in the case of styrene (Chapter III), where radiolabelling with ^{14}C at carbon number 8 in the side chain, the carbon which is split off during the biotransformation of styrene, gave a very different distribution pattern of firmly bound styrene metabolites compared to radiolabelling with ^{14}C in the aromatic ring.

ELIMINATION STUDIES

The purpose of the elimination studies of the solvents selected for autoradiography was primarily to add some quantitative information about their fate in the body. Thus, since the same amount of the solvents was inhaled by the animals for autoradiography as for the elimination studies, the elimination

studies gave valuable information on the dose administered also for autoradiography as well as data on the time course and quantitative elimination of the solvents and their metabolites via various routes. Secondly, the data were subject to mathematical and computerized analysis in an attempt to correlate the time course of elimination of unchanged solvents in the exhaled air with the autoradiographic results and to compare the individual compounds within the two groups of solvents. The attempt proved to be most successful in the case of the aromatic solvents whereas the results were not as easily interpreted in the case of some of the chlorinated solvents. The results of the computerized compartmental analysis demonstrate that pharmacokinetic theories may be applied with success also in animal experimentation with organic solvents, which has hitherto occurred only rarely, and also with the help of respiratory elimination data in addition to previously used tissue and blood concentration data (185, 474).

S U M M A R Y

The present investigation was done with the aim to study the distribution, specific localizations and retention of some selected organic solvents and their metabolites in the living organism after an inhalational exposure. For this purpose modified whole-body autoradiographic techniques were applied. Autoradiography at low temperature (-80°C) according to previously developed techniques (Chapter I) was used to register the total radioactivity including the volatile, non-metabolized part of the solvents. The autoradiograms obtained at low temperature were compared to autoradiograms of dried, evaporated sections showing the distribution of non-volatile metabolites of the solvents. In addition, a third autoradiographic technique was developed which allowed the registration of firmly bound, non-volatile solvent metabolites from dried, evaporated and extracted whole-body sections (Chapter I).

In a second facet of the investigation an attempt was made to quantitatively determine the uptake and elimination of the solvents. The expiration of volatile, non-metabolized solvents and possible volatile metabolites (CO_2 and CO) was quantified and followed with time with the help of a specially constructed metabolic cage (Chapter I). The time course of expiration of the unchanged part of the solvents was subject

to computerized pharmacokinetic analysis (Chapter I) to obtain additional information on the transfer of the solvents in the body.

Benzene, toluene, xylene and styrene were chosen as representatives of the aromatic hydrocarbon solvents. Methylene chloride, chloroform, carbon tetrachloride and trichloroethylene represented the chlorinated aliphatic hydrocarbon solvents.

As evidenced by low-temperature autoradiography, all solvents showed a high affinity for nervous and adipose tissues where they were rapidly taken up after inhalation. The retention time of the solvents in these tissues was dependent on their fat solubility (Appendix 2). Accordingly, styrene showed the longest retention time in nervous and fatty tissues among the aromatic solvents (Chapter III) whereas carbon tetrachloride was retained in nervous and fatty tissues for a longer period of time than any other chlorinated solvent (Chapter IV). However, a notable exception was found in the case of chloroform (Chapter IV), which showed a long-time, specific retention in the cerebellar cortex, meninges and spinal nerves. The fat solubility of the solvents was also found to influence their pharmacokinetics since lower rate constants were generally obtained for the expiration of more fat soluble solvents.

All solvents were rapidly biotransformed with metabolites appearing mainly in the liver and kidney within a very short time after inhalation. An accumulation of non-volatile radioactivity in these organs was noted at short survival times. Metabolites were also localized to the bronchi of the lung after inhalation of benzene, xylene (Chapter II), styrene

(Chapter III) and all chlorinated solvents (Chapters IV and V). A specific localization of non-volatile metabolites to the testicular interstitium was noted after inhalation of benzene (Chapter II), chloroform and carbon tetrachloride (Chapter IV). A high concentration of non-volatile metabolites was also registered in the gastro-intestinal mucosa after inhalation of carbon tetrachloride (Chapter IV). No non-volatile radioactivity was registered in nervous or fatty tissues. Metabolites of all solvents were excreted both via urine and bile. Within the time of the autoradiographic study, 48 hours, only toluene and xylene and their metabolites were completely eliminated from the body (Chapter II). Metabolites of toluene and xylene, although completely extractable, could be registered still 8 and 24 hours respectively after inhalation. The longer retention time of xylene and its metabolites was probably due to the higher fat solubility of xylene as compared to toluene and its consequently slower release from the fat depots of the body and more extended availability for biotransformation. In the case of all other solvents firmly bound, non-volatile radioactivity was registered in a number of tissues still 48 hours after inhalation, probably due partly to the breakdown of the solvents to one-carbon fragments incorporated into tissues via normal metabolic pathways, but also due to the binding of reactive solvent intermediates or metabolites to various tissue components. The liver, kidney as well as the bronchi of the lung were registered as the main target tissues for these reactive solvent biotransformation products.

Three-compartment pharmacokinetics were observed for the majority of the solvents selected for study although a two-

compartment model was found to fit the elimination rate curves for styrene, methylene chloride and chloroform in the exhaled air. CO_2 was identified as a metabolite of benzene (Chapter II), styrene (Chapter III), methylene chloride, chloroform and carbon tetrachloride (Chapter IV) and trichloroethylene (Chapter V) whereas only methylene chloride was biotransformed to CO (Chapter IV).