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Meeting Announcement

The 8th European Workshop on Drug Metabolism will be held at the University of Liège, Belgium, September 5–9, 1982.

For further information, please contact: Professor Jacques E. Gielen, Laboratoire de Chimie Médicale, Institut de Pathologie, Université de Liège, Bâtiment B 23, B-4000 Sart Tilman par Liège 1, Belgium, telephone (32-41)-56.24.80/81.

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Original Investigations

Inhalation Pharmacokinetics Based on Gas Uptake Studies

III. A Pharmacokinetic Assessment in Man of "Peak Concentrations" of Vinyl Chloride

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Abstract. On the basis of previous determinations of pharmacokinetic parameters for inhaled vinyl chloride in men, rhesus monkeys, and rats, and on improved pharmacokinetic models a pharmacokinetic treatment of the problem of "peak concentrations" of vinyl chloride, as occuring in industrial practice, became possible. For the calculations, metabolic elimination kinetics of vinyl chloride was assumed to be first order as experiments in different species including rhesus monkeys showed "linear" pharmacokinetics up to atmospheric exposures of 200–300 ppm. The distribution of vinyl chloride between atmosphere and organism under different conditions was evaluated using "steady-state-kinetics". After treating the processes of "influx", "efflux", and "metabolism", the numerical values for the parameters derived from a human kinetic experiment were used to theoretically calculate the time courses of concentration of vinyl chloride in the organism and of the cumulative amount of vinyl chloride metabolized, under the conditions of (a) a 2 h constant exposure to 5 ppm vinyl chloride and (b) two subsequent "peaks" of 50 ppm with a duration of 5 min each. This model calculation suggested that, regardless of the exposure profile, the amount of (reactive) metabolites formed from vinyl chloride would solely be a function of the mean atmospheric vinyl chloride concentration over time. The general validity of this suggested rule could subsequently be demonstrated. As the concentration of the reactive metabolite of vinyl chloride responsible for the carcinogenic effect at the target site must be a resultant of both formation and inactivation, an evaluation of the differential risk of different exposure profiles can reasonably be based on biochemical examinations of the "detoxifying" pathways. This points out the relevance of studies of the patterns of different metabolites of vinyl chloride in man under varying exposure profiles.

Key words: Vinyl chloride – Pharmacokinetics – Peak concentration – Inhalation kinetics

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Introduction

Considerable efforts have been directed towards developing a general concept for the limitation of "peak concentrations" of hazardous chemicals at the workplace. Proposals have been elaborated for non-carcinogenic compounds (Henschler 1979) which are based on both pharmacokinetic grounds and classical dose-response considerations. For carcinogens, a sound scientific basis for such an assessment is lacking so far. To promote a further discussion of this matter, the present paper describes one part of the situation, from a pharmacokinetic viewpoint, dealing with an outstanding example, vinyl chloride.

There is now sufficient evidence that the carcinogenic principle of vinyl chloride is a reactive metabolite, most probably chloroethylene oxide, another reactive metabolite is the product of spontaneous rearrangement of this epoxide, chloroacetaldehyde (Guengerich et al. 1979; Zajdela et al. 1980; Bolt et al. 1980). Because the initial step of metabolism of vinyl chloride is epoxidation by (microsomal) monooxygenases, this means that the overall metabolic elimination of vinyl chloride which can be determined in vivo by pharmacokinetic methods, must be equivalent to the total amount of epoxide produced. Interesting proposals have been published (Gehring et al. 1978, 1979; Anderson et al. 1980) to incorporate pharmacokinetic data of vinyl chloride into calculations of dose-responses and into risk assessment. Such models imply, either explicitly or tacitly, that the response (cancer development) is a function of reactive metabolites produced over time, rather than a function of plain atmospheric vinyl chloride exposure.

Hence, calculations of the actual amounts of vinyl chloride metabolized by the human organism under various conditions of exposure, upon careful observation of the implicit limitations, could give some idea of the risk of these exposures, and could possibly contribute to tackle the outstanding problems mentioned above. Such calculations can now be based on expanded models for inhalation pharmacokinetics (Filser and Bolt 1981) and on appropriate experimental data for vinyl chloride in rats (Filser and Bolt 1979), rhesus monkeys (Buchter et al. 1980), and in man (Buchter et al. 1978; Buchter 1979).

In the United States, a threshold limit value ("TLV") of 5 ppm has recently been adopted for vinyl chloride (ACGIH 1980). In the Federal Republic of Germany, a "TRK" value of 2 ppm (or 5 ppm for pre-existing plants) is in force (see DFG 1980). Other countries have similar regulations. However, it is well documented that in industrial practice multiple peak concentrations do occur which by far exceed 5 ppm vinyl chloride (Baker and Reiter 1977). Most of these peaks (which may occur several times a day) have a duration of a few minutes only; individual peaks may even reach concentrations of 50 ppm and more. Because the influx of vinyl chloride into the organism is a time-dependent process, it was suggested (Buchter et al. 1978) that very short single peaks did not considerably contribute to the overall load of vinyl chloride metabolites in the organism when compared to a constant exposure to the allowable "TLV" or "TRK". However, a quantitative treatment was not possible at that time because of the lack of appropriate pharmacokinetic tools.

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Pharmacokinetic Model

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The present paper describes, under various conditions, the rates of metabolic elimination of vinyl chloride from the human organism which, according to what has been mentioned above, is equivalent to the rates of epoxide metabolically produced. This now provides part of the basis for a scientific evaluation of the problem of varying exposure profiles of vinyl chloride.

Pharmacokinetic Model

First of all, the non-linearity of pharmacokinetics of vinyl chloride must be observed while selecting a kinetic model. In rats, metabolic elimination of vinyl chloride follows first-order kinetics if atmospheric exposure does not exceed 250 ppm (Filser and Bolt 1979). Above this point the metabolizing capacities become saturated. The theoretical implications of this phenomenon have been discussed in detail (Filser and Bolt 1981). In rhesus monkeys, a species displaying a quantitative metabolic pattern of vinyl chloride much similar to man, the same saturation point of vinyl chloride metabolism was observed (Buchter et al. 1980), and metabolic elimination again followed a first-order rule up to atmospheric vinyl chloride concentrations of 200–300 ppm. Because of the carcinogenicity of vinyl chloride human experimentation in this dose range, to verify this saturation pattern, is not possible. However, the close agreement of data obtained from rats and from a primate species in this particular point suggests that also in man linear pharmacokinetics of vinyl chloride which are in fact observed at very low exposure concentrations (Buchter et al. 1978) should be valid up to atmospheric concentrations of 200–300 ppm. Because this is entirely the range within which "peak concentrations" do occur in industrial practice, we decided to exclusively base our calculations on a linear pharmacokinetic model, bearing in mind the implicit limitation that this is valid for exposures up to 200–300 ppm only.

Pharmacokinetic Data in Man

The basic pharmacokinetic data on vinyl chloride in man have been previously published (Buchter et al. 1978; Buchter 1979); the present analyses are based on this material. At low vinyl chloride concentrations, experiments both in a "closed" and in an "open" system (where concentrations in inhaled and exhaled air were monitored) gave similar metabolic clearance values for the disappearance of vinyl chloride from the air. These data also demonstrated a considerable interindividual variation in the distribution behaviour of vinyl chloride in man which, according to animal experiments (Buchter et al. 1977) ought to be due to a differing mass of adipose tissue. Therefore, we decided to base all the specific numeric calculations of this report on the actual pharmacokinetic parameters determined in one individual only (subject "B": Buchter et al. 1978; Buchter 1979).

By analogy to the denominations of Filser and Bolt (1981), the model and conditions of the experiment in question (Buchter et al. 1978; Buchter 1979) are

summarized in Fig. 1a. In the closed atmosphere of 8 l (volume of spirometer containing air/vinyl chloride *plus* the residual breath volume) the actual decline of vinyl chloride given in Fig. 2a was measured. This decline followed the general function

$$y_{100} = (y_{100} - y'_{100})\, e^{-\delta t} + y'_{100} \cdot e^{-k' t} \qquad (1)$$

whereby "y'_{100}" is obtained by extrapolation of the second decline phase to $t = 0$.

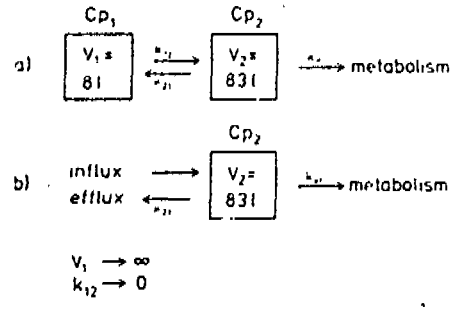

Fig. 1a and b. Block diagram of pharmacokinetic systems for inhalation of vinyl chloride (numerical values of subject B., Buchter et al. 1978): (a) inhalation from a closed gas phase as described in Buchter et al. 1978 and Buchter 1979; (b) translation of pharmacokinetic parameters into an "open" system of exposure

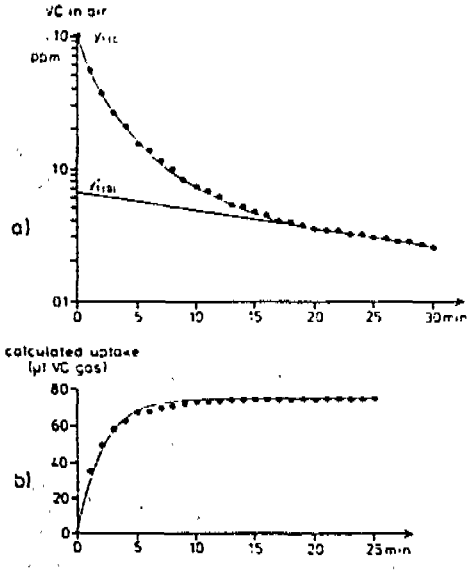

Fig. 2a and b. Determination of pharmacokinetic parameters for vinyl chloride in man: (a) experimental data obtained with subject B (Buchter et al. 1978; Buchter 1979) in the air phase (Cp_1) of the system shown in Fig. 1a, and extrapolation of the final logarithmic decline to $t = 0$ [y'_{100}]; (b) demonstration of the initial process of "equilibration", values being calculated from the experimental data of the first section of the curve in panel (a) minus the corresponding points on the curve extrapolated from the second phase, expressed as calculated gas uptake of vinyl chloride (VC)

The numerical v

$\delta = 29.7\ h^{-1}$;

$k' = 1.90\ h^{-1}$.

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$$K = \frac{y'_{100}}{y'_{100}} = \frac{(y_{100}}{y'_1}$$

We obtained $K = 1.35$
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Bolt, 1981), k_{el} (Fig. 1
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$$k_{el} = \frac{Cl_{tot}}{V_2 K_{st}}$$

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$k_{12} \gg k_{21}, k_{el}$

The numerical values of the rate constants were:

$\delta = 29.7\ h^{-1}$;

$k' = 1.90\ h^{-1}$.

These values were obtained by using a log regression calculator program. The highest coefficient of correlation ($r = 0.9913$) for the second decline process was obtained when the experimental points after $t = 20$ min were taken for that calculation. These results differ slightly from those of a preliminary, merely graphical, evaluation of the decline curve (Buchter et al. 1978; Buchter 1979).

From the values of $y_{1(0)}$, $y'_{1(0)}$, the volumes of the air phase (V_1) and the organism (V_2) the distribution of vinyl chloride was calculated which actually prevailed under the conditions of the experiment (Buchter 1979):

$$K = \frac{y'_{2(0)}}{y'_{1(0)}} = \frac{(y_{1(0)} - y'_{1(0)})V_1}{y'_{1(0)}V_2}. \tag{2}$$

We obtained $K = 1.35$. This K derived here is *not* identical with the "equilibrium constant" (Filser and Bolt 1979)

$$K_{eq} = \frac{k_{12}V_1}{k_{21}V_2} = \frac{y_{2(\infty)}}{y_{1(\infty)}} \tag{3}$$

which is derived under theoretical conditions of a static equilibrium with negligible metabolism. The conditions of distribution between Cp_2 and Cp_1 with concurrent metabolic elimination may appropriately be described by introducing the "steady state constant", K_{st}, as derived by Filser and Bolt (1981: Eq. 14). Therefore, K (Eq. 2) can be regarded as an experimentally obtained value for K_{st} (not for K_{eq}). From K_{st} and the metabolic clearance Cl_{tot} (Eq. 22 of Filser and Bolt, 1981), k_{el} (Fig. 1) can be calculated by using Eq. (26) of Filser and Bolt (1981):

$$k_{el} = \frac{Cl_{tot}}{V_2 K_{st}}. \tag{4}$$

The numerical value for subject B is: $k_{el} = 2.03\ h^{-1}$. Of the kinetic constants (Fig. 1a) we still do not know k_{12} and k_{21}. The rate constant of the fast process of the experimental curve in Fig. 2 (a and b), δ, is determined by all the three rate constants of the partial processes involved, i.e., k_{12}, k_{21}, and k_{el} (Glantz 1979). An exact solution of this problem is complicated; it is included in the Appendix to this paper as Eq. (39). However, under the assumption

$$k_{12} \gg k_{21}, k_{el} \tag{5}$$

the system simplifies into:

$$\delta \approx k_{12} . \tag{6}$$

Then k_{12} would amount to

$$k_{12} = 29.7\ h^{-1} .$$

(The solution derived in the Appendix results in $k_{12} = 27.8\ h^{-1}$.)

To obtain k_{21}, rearrangement of Eq. (19) of Filser and Bolt (1981) gives

$$k_{21} = \frac{V_1(k_{12} - k')}{V_2 K_{ai}} ; \tag{7}$$

hence, we obtain

$$k_{21} = 1.98\ h^{-1} .$$

(Again, the solution given in the Appendix results in a similar value: $k_{21} = 1.77\ h^{-1}$.)

Fig. 3a–d. Kinetic implicatio
constant exposure to low lev
chloride (5 ppm for 2 h): (a)
of atmospheric exposure, y_1;
(b) calculated concentration
organism, y_2; (c) calculate
dose metabolized, N_{ei}; (d) c
cumulative dose eliminated t
of unmetabolized vinyl chlori

Translation of the Pharmacokinetic Data into "Open" Exposure Conditions

After obtaining all the pharmacokinetic data for vinyl chloride in man under the conditions of the experimental system of Fig. 1a, a transition is necessary to the conditions of inhalation of the compound from an "open" atmosphere, i.e., $V_1 \rightarrow \infty$ (Fig. 1b). Because the clearance term $k_{12} V_1$ (see Eqs. 2 and 3 of Filser and Bolt 1981) remains constant, this transition also implies $k_{12} \rightarrow 0$. Both k_{ei} and k_{21} remain unchanged. Also, it must be observed that K_{ai} is dependent on the ratio V_1/V_2. According to Eq. (36) of Filser and Bolt (1981), K_{ai} of subject B can be calculated for $V_1 \rightarrow \infty$ to be

$$K_{ai} = 0.714$$
$$(V_1 \rightarrow \infty) .$$

For calculation of metabolic rates, we need knowledge of the actual concentrations in Cp_2 (Eq. 24 of Filser and Bolt, 1981). This is $K_{ai} \cdot y_1$ when the final concentration in Cp_2 is reached on a continous atmospheric exposure to constant vinyl chloride levels. However, when regarding rapidly changing "peak concentrations" of exposure, the actual concentration in Cp_2 is determined by all the three processes of "influx", "efflux", and "metabolism" (Fig. 1b).

In order to simulate realistic "peak concentration" conditions, we calculated the actual concentration in Cp_2 as well as the cumulative dose of vinyl chloride metabolized for single clear-cut long-term and short-term exposures, as

Fig. 4a–c. Kinetic implicatio
intermittent exposure to "peak
concentrations" of vinyl chlori
(example of two peaks of 50 p
5 min each): (a) conditions of
atmospheric exposure, y_1; (b)
concentration in the organism,
(c) calculated cumulative dose
metabolized, N_{ei}

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$k_{21} = 1.77$

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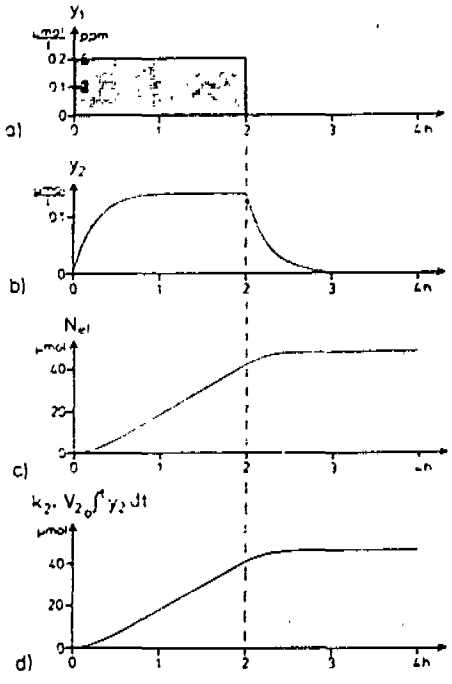

Fig. 3a–d. Kinetic implications of a *constant exposure* to low levels of vinyl chloride (5 ppm for 2 h): **(a)** conditions of atmospheric exposure, y_1; **(b)** calculated concentration in the organism, y_2; **(c)** calculated cumulative dose metabolized, N_{el}; **(d)** calculated cumulative dose eliminated by exhalation of unmetabolized vinyl chloride

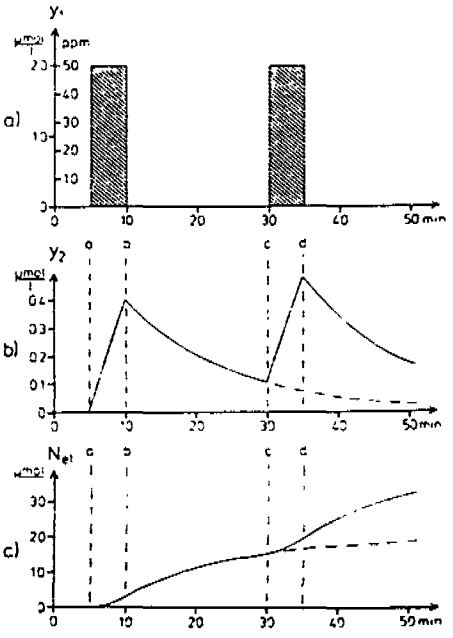

Fig. 4a–c. Kinetic implications of an *intermittent exposure* to "peak concentrations" of vinyl chloride (example of two peaks of 50 ppm for 5 min each): **(a)** conditions of atmospheric exposure, y_1; **(b)** calculated concentration in the organism, y_2; **(c)** calculated cumulative dose metabolized, N_{el}

indicated in Fig. 3 and Fig. 4. This calculation was based on the following considerations.

The *"influx"* (Fig. 1b) which is determined by Eqs. (2) and (3) of Filser and Bolt (1981), remains constant as y_1 remains at a constant value [i.e., y_1 is fixed as $y_{1(\infty)}$: see Eq. (3)]:

$$\frac{dN_{1\,2}}{dt} = -k_{21}K_{eq}V_2y_1 = -\frac{dN_{2\,1}}{dt}. \tag{8}$$

The *"efflux"* (Fig. 1b) is described by Eq. (4) and (5) of Filser and Bolt (1981):

$$\frac{dN_{2\,21}}{dt} = -k_{21}V_2y_2. \tag{9}$$

The *"metabolism"* (Fig. 1b), according to Eq. (24) of Filser and Bolt (1981), is:

$$\frac{dN_{el}}{dt} = -k_{el}V_2y_2. \tag{10}$$

The change in the amount of vinyl chloride being in Cp_2 is the resultant of Eqs. (8–10):

$$\frac{dN_2}{dt} = V_2\frac{dy_2}{dt} = K_{eq}k_{21}V_2y_1 - k_{21}V_2y_2 - k_{el}V_2y_2. \tag{11}$$

This differential equation is solved and divided by V_2:

$$y_{2(t)} = K_{eq}y_1\frac{k_{21}}{k_{21}+k_{el}}\left[1 - e^{-(k_{21}+k_{el})t}\right]. \tag{12}$$

This equation describes the ascending section of the concentration curve in Cp_2 (from a to b in Fig. 4b). After ending the atmospheric "peak concentration", y_1 in Eq. (8) becomes zero, and the $y_{2(b)}$ reached according to Eq. (12) at that time declines, due to the partial processes described in Eqs. (9) and (10). Hence, the course of Fig. 4b subsequent to point *b* is:

$$y_{2(t)} = y_{2(b)} \cdot e^{-(k_{21}+k_{el})t}. \tag{13}$$

If a second "peak concentration" occurs at a later time point (*c*) where residual vinyl chloride is still present in Cp_2, some cumulation occurs as indicated in Fig. 4b.

The amount of vinyl chloride metabolized under such conditions is shown in Fig. 4c. It is given by the differential Eq. (10). For the section between time points *a* and *b* (Fig. 4c) this is solved into

$$\int_a^t dN_{el} = -k_{el}$$

or

$$N_{el(t)} = V_2K_{eq}y_1$$

After time point *b*, present in Cp_2:

$$\int_b^t dN_{el} = k_{el}V_2\int_b^t$$

The amount metabo is:

$$N_{el(t)} = V_2y_{2(b)}\frac{}{k_2}$$

The Simulation of I

Following the above of y_2, (*c*) the cumul cumulative dose expi theoretically exposed vinyl chloride (*a*). Th via metabolism and v k_{el} and k_{21} in this sul during and after this

By contrast, in Fi of 5 min each, and an that current technical an alarm signal to be trations at the works. amount of vinyl chlori (see Fig. 4c) was 40 µ (panel b) and of the

The results of th $5 \times 120 = 600$ ppm × (Fig. 3): the intermitte in the same figure of suggest that, regardle formed from vinyl chlo

$$\int_{a}^{b} dN_{el} = -k_{el}V_2 \cdot \int_{a}^{b} y_2 dt\,, \tag{14}$$

or

$$N_{el(a)} = V_2 K_{ex} y_1 \frac{k_{el}k_{21}}{k_{el} + k_{21}} \left[\frac{1}{k_{21} + k_{el}}(1 - e^{-(k_{el} + k_{21})t}) - t\right]. \tag{15}$$

After time point b, further metabolism occurs because vinyl chloride is still present in Cp_2:

$$\int_{b}^{t} dN_{el} = k_{el}V_2 \int_{b}^{t} y_2 dt\,. \tag{16}$$

The amount metabolized during the decline phase in Cp_2, up to a time point t, is:

$$N_{el(t)} = V_2 y_{2(b)} \frac{k_{el}}{k_{21} + k_{el}} [e^{-(k_{el} + k_{21})t} - 1]\,. \tag{17}$$

The Simulation of Peak Concentrations

Following the above ways of calculation Fig. 3 demonstrates (*b*) the time course of y_2, (*c*) the cumulative vinyl chloride dose metabolized (N_{el}), and (*d*) the cumulative dose expired, if subject B (Buchter et al. 1978; Buchter 1979) were theoretically exposed for 2 h to the current TLV/TRK concentration of 5 ppm vinyl chloride (*a*). The doses of vinyl chloride eliminated under these conditions via metabolism and via exhalation are very similar, because of the similarity of k_{el} and k_{21} in this subject. The overall amount of vinyl chloride metabolized during and after this (theoretical) exposure was 48 µmol.

By contrast, in Fig. 4 analogous computations for two peaks concentrations of 5 min each, and amounting to 50 ppm, were made. It should be mentioned that current technical regulations in the Federal Republic of Germany provide an alarm signal to be given when actual atmospheric vinyl chloride concentrations at the worksite exceed the "TRK" for more than 5 min. The total amount of vinyl chloride metabolized during and after the two peaks of 50 ppm (see Fig. 4c) was 40 µmol. Figure 4 demonstrates both the time course of y_2 (panel b) and of the cumulative amount metabolized (panel c).

The results of the two calculations show that a constant exposure of $5 \times 120 = 600$ ppm × min led to 0.08 µmol per atmospheric ppm per minute (Fig. 3); the intermittent exposure (Fig. 4) of $50 \times 10 = 500$ ppm × min resulted in the same figure of 0.08 µmol per atmospheric ppm per min. This would suggest that, regardless of the exposure profile, the amount of metabolites formed from vinyl chloride would solely be a function of the mean vinyl chloride

concentration over time. If this were true, the amount of vinyl chloride metabolized after ending an exposure should equal the difference of metabolism between the theoretical case of steady-state and that amount actually metabolized up to arriving at the steady-state (or up to the end of exposure). By use of an analog computer, this has already been shown for a series of organic solvents by Droz and Fernandez (1977).

The general validity of this rule is demonstrated by the following calculations. Equation (15) gives the metabolized amount during exposure, Eq. (17) that after exposure. The sum of both, $N_{el(tot)}$, represents the total amount metabolized during and after exposure. Hereby, $y_{2(b)}$ in Eq. (17) is to be replaced by the expression of Eq. (12). Also, to obtain the entire amount metabolized, the t in Eq. (17) must go towards ∞. The result is:

$$N_{el(tot)} = -\frac{k_{el}k_{21}}{k_{21} + k_{el}} K_{eq} V_2 y_1 t \text{ peak} \tag{18}$$

whereby t peak is the duration of the exposure peak and y_1 its (mean) concentration. The connection of K_{eq} and K_a under conditions of exposure to an open atmosphere is given by Eq. (3) of this paper and by Eq. (36) of Filser and Bolt (1981).

Hence, it follows that

$$N_{el(tot)} = -K_a y_1 V_2 k_{el} \cdot t \text{ peak} . \tag{19}$$

According to the definition of K_a (Eq. 7 in Filser and Bolt 1981), this can be written

$$N_{el(tot)} = -y_2 V_2 k_{el} \cdot t \text{ peak} \tag{20}$$

which, according to Eq. (24) of Filser and Bolt (1981), determines that amount which is metabolized during a time t after previous formation of steady-state conditions.

Thus, it is clear that *the mean exposure concentration over time is the only determinant for the amount of vinyl chloride metabolized*, also under varying exposure profiles.

Discussion

The carcinogenicity of vinyl chloride is the principle toxic effect which is relevant for the regulations covering the handling of this compound. This effect is due to reactive metabolites, most probably chloroethylene oxide, as mentioned in the Introduction section. A quantitative treatment of the comparative risk of different exposure profiles must consider that the actual amount or concentration of chloroethylene oxide in the human organism is always a resultant of both formation by microsomal monooxygenases and breakdown by "detoxifying pathways". The concen the time factor should b consequence, for the h (Bolt et al. 1981a).

The present data cl exposures vs. peak con chloride metabolized a epoxide formed is only concentration over time provided they do not ex vinyl chloride metaboli tion).

Because the concent by formation and by re present data that an a exposure to vinyl chlor processes and pathways principally by using bio

A possible way of tac et al. (1980). After ra S-hydroxyethyl-N-acetyl of the epoxide, in cont demonstrated a differe exposure conditions. Th direct action of glutathi characteristic species as glutathione-S-transferase Bolt et al. 1981b) sugges the capacity for a direct with glutathione is limite possible immediate detox of vinyl chloride could p peaks which produce metabolite.

Hence, necessary re encouraged by our pres metabolite: a future ass chloride exposure profile detoxication.

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Appendix

An exact way to a deter

The partial process fr Filser and Bolt (1981); sin

pathways". The concentration of the reactive metabolite built up in the liver and the time factor should be critical for the extent of alkylation at DNA sites and, in consequence, for the hepatic tumorigenic potential evoked by the xenobiotic (Bolt et al. 1981a).

The present data clearly show that the exposure profile (e.g., continous low exposures vs. peak concentrations) does not determine the net amount of vinyl chloride metabolized and hence the amount of epoxide formed: the amount of epoxide formed is only a function of the integral of the atmospheric exposure concentration over time. This is valid also for very short concentration peaks, provided they do not exceed the range of 200–300 ppm where saturation of the vinyl chloride metabolizing enzyme system must be anticipated (see Introduction).

Because the concentration of vinyl chloride-derived epoxide is determined by formation and by removal (elimination) of this epoxide, it follows from the present data that an evaluation of differential risks of different profiles of exposure to vinyl chloride may reasonably be focussed only on the different processes and pathways by which the epoxide is eliminated. This must be done principally by using biochemical methodologies.

A possible way of tackling this problem has recently been outlined by Müller et al. (1980). After suggesting that one urinary vinyl chloride metabolite, S-hydroxyethyl-N-acetylcysteine, is formed directly by glutathione conjugation of the epoxide, in contrast to another metabolite, thiodiglycolic acid, they demonstrated a differential excretion of both metabolites under differing exposure conditions. The validity of the underlying assumption of a possible direct action of glutathione on the epoxide could be proven by establishing characteristic species differences in hepatic microsomal (membrane-bound) glutathione-S-transferases (Bolt et al. 1981b). These data (Müller et al. 1980; Bolt et al. 1981b) suggest that in man, much in contrast to the situation in rats, the capacity for a direct detoxication of the epoxide formed from vinyl chloride with glutathione is limited only. This would indicate that, from the standpoint of possible immediate detoxication of the epoxide, low but constant exposure levels of vinyl chloride could possibly be less hazardous than marked concentration peaks which produce just the same integral amounts of the reactive metabolite.

Hence, necessary further studies on this latter point are very much encouraged by our present evaluation of the kinetics of formation of the metabolite; a future assessment of the differential hazard of different vinyl chloride exposure profiles can be based almost entirely on the biochemistry of detoxication.

Appendix

An exact way to a determination of the rate constants k_{12}, k_{21}, and k_{el}.

The partial process from Cp_1 into Cp_2 (see Fig. 1a) is given by Eq. (2) of Filser and Bolt (1981); similarly, Eq. (4) of Filser and Bolt (1981) describes the

partial process from Cp_2 into Cp_1. Hence, the change of mass of compound in Cp_1 with time,

$V_1 \frac{dy_1}{dt}$, is

$$V_1 \frac{dy_1}{dt} = -k_{12}V_1y_1 + k_{21}V_2y_2 . \tag{21}$$

The change in Cp_2 is influenced by the three partial processes determined by k_{12}, k_{21}, and k_{el} (Fig. 1a):

$$V_2 \frac{dy_2}{dt} = +k_{12}V_1y_1 - (k_{12} + k_{el})V_2y_2 . \tag{22}$$

In this equation, V_2y_2 may be replaced by

$$V_2y_2 = \frac{\frac{dy_1}{dt}V_1 + k_{12}V_1y_1}{k_{21}} \tag{23}$$

which is derived from Eq. (21).

Differentiation of Eq. (21) gives

$$V_1 \frac{d^2y_1}{dt^2} = -k_{12}V_1 \frac{dy_1}{dt} + k_{21}V_2 \frac{dy_2}{dt} . \tag{24}$$

Equation (24), with Eqs. (22) and (23), leads to

$$\frac{d^2y_1}{dt^2} + (k_{12} + k_{21} + k_{el}) \frac{dy_1}{dt} + k_{12}k_{el}y_1 = 0 \tag{25}$$

This is a homogenous second order differential equation. The fundamental equation

$$\lambda^2 + (k_{12} + k_{21} + k_{el})\lambda + k_{12}k_{el} = 0 \tag{26}$$

has roots

$$\lambda_1 = \frac{-(k_{12} + k_{21} + k_{el}) - \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{12}k_{el}}}{2} \tag{27a}$$

and

$$\lambda_2 = \frac{-(k_{12} + k_{21} + k_{el}) + \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{12}k_{el}}}{2} . \tag{27b}$$

The general solution

$$y_1 = C_1 \cdot e^{\lambda t} + C$$

This means that, at

$$y_{1(0)} = C_1 + C_2 .$$

Differentiation of E

$$\frac{dy_1}{dt} = \lambda_1 C_1 e^{\lambda_1 t} + \lambda$$

Because obviously $t = 0$:

$$\frac{dy_{1(0)}}{dt} = -k_{12}y_{1(0)}$$

From Eqs. (29) and

$$C_1 = \frac{y_{1(0)}(\lambda_2 + k_1}{\lambda_2 - \lambda_1}$$

From this equation, (27b):

$$C_1 = y_{1(0)} \frac{k_{12} - (k}{}$$

From Eqs. (29) and

$$C_2 = \frac{y_{1(0)}(k_{12} + \lambda_1}{\lambda_1 - \lambda_2}$$

Again, λ_1 and λ_2 are

$$C = y_{2(0)} \frac{k_{21} + k_{el}}{}$$

From Eq. (28) of Fil

$$(k')^2 - k'(k_{12} + k_2$$

ipound in

The general solution of Eq. (25) is

$$y_1 = C_1 \cdot e^{\lambda_1 t} + C_2 \cdot e^{\lambda_2 t}. \tag{28}$$

This means that, at $t = 0$,

(21)

$$y_{1(0)} = C_1 + C_2. \tag{29}$$

ed by k_{12}.

Differentiation of Eq. (28) gives

$$\frac{dy_1}{dt} = \lambda_1 C_1 e^{\lambda_1 t} + \lambda_2 C_2 e^{\lambda_2 t}. \tag{30}$$

(22)

Because obviously $y_{2(0)}$ is zero, it follows from Eqs. (21) and (30) for $t = 0$:

$$\frac{dy_{1(0)}}{dt} = -k_{12} y_{1(0)} = \lambda_1 C_1 + \lambda_2 C_2. \tag{31}$$

(23)

From Eqs. (29) and (31) we obtain

$$C_1 = \frac{y_{1(0)}(\lambda_2 + k_{12})}{\lambda_2 - \lambda_1}. \tag{32}$$

(24)

From this equation, λ_1 and λ_2 are eliminated by inserting Eqs. (27a) and (27b):

$$C_1 = y_{1(0)} \frac{k_{12} - (k_{21} + k_{e1}) + \sqrt{(k_{12} + k_{21} + k_{e1})^2 - 4 k_{12} k_{e1}}}{2\sqrt{(k_{12} + k_{21} + k_{e1})^2 - 4 k_{12} k_{e1}}}. \tag{33}$$

(25)

lamental

From Eqs. (29) and (31) we obtain for C_2:

$$C_2 = \frac{y_{1(0)}(k_{12} + \lambda_1)}{\lambda_1 - \lambda_2}. \tag{34}$$

(26)

Again, λ_1 and λ_2 are eliminated:

$$C_2 = y_{1(0)} \frac{k_{21} + k_{e1} - k_{12} + \sqrt{(k_{12} + k_{21} + k_{e1})^2 - 4 k_{12} k_{e1}}}{2\sqrt{(k_{12} + k_{21} + k_{e1})^2 - 4 k_{12} k_{e1}}}. \tag{35}$$

(27a)

From Eq. (28) of Filser and Bolt (1981) is follows that

$$(k^*)^2 - k^*(k_{12} + k_{21} + k_{e1}) + k_{12} k_{e1} = 0. \tag{36}$$

(27b)

Comparison of Eqs. (36), (26), and (27b) reveals that

$$-\lambda_2 = k' , \tag{37}$$

(see Eq. 1).

Then, according to Eq. (27a), $-\lambda_1$ represents the rate constant of the last process in Eq. (1):

$$-\lambda_1 = \delta , \tag{38}$$

A combination of Eqs. (33), (35), (37), (38), and Eq. (28) gives the exact formulation of y_1:

$$y_1 = \frac{y_{1(0)}[k_{12} - (k_{21} + k_{e1}) + \sqrt{(k_{12} + k_{21} + k_{e1})^2 - 4\,k_{12}k_{e1}}]}{2\sqrt{(k_{12} + k_{21} + k_{e1})^2 - 4\,k_{12}k_{e1}}} \cdot e^{-\lambda_2 t}$$

$$+ \frac{y_{1(0)}[k_{21} + k_{e1} - k_{12} + \sqrt{(k_{12} + k_{21} + k_{e1})^2 - 4\,k_{12}k_{e1}}]}{2\sqrt{(k_{12} + k_{21} + k_{e1})^2 - 4\,k_{12}k_{e1}}} \cdot e^{-\lambda_1 t} . \tag{39}$$

Figure 2a shows that the fast exponential process, determined by the rate constant δ, is practically completed after 20 min (see above). At $t = 20$ min (Fig. 2a) $y_{(t)}$ was about 0.35 ppm vinyl chloride; the contribution of the first exponential partial function in Eq. (39) at that time point, is $< 4.7 \times 10^{-4}$ ppm. The further course of y_1 is, therefore, determined by the second exponential partial function in Eq. (39) only, and the extrapolation to $y'_{1(0)}$ (Fig. 2a) is justified. This means, for practical reasons,

$$C_2 = y'_{1(0)} \tag{40}$$

which was 0.668 ppm in the experiment of Fig. 2a. Hence, it follows (Eqs. 29 and 40):

$$C_1 = y_{1(0)} - y'_{1(0)} \tag{41}$$

which was 9.332 ppm in the experiment.

For determination of the rate constants of the partial processes (k_{12}, k_{e1}, k_{21}) the following calculations are made: Eqs. (29) and (31) are rearranged to give

$$k_{12} = -\frac{\lambda_1 C_1 + \lambda_2 C_2}{C_1 + C_2} \tag{42}$$

which is, with Eqs. (29), (37), (38), (40), and (41)

$$k_{12} = \frac{\delta(y_{1(0)} - y'_{1(0)}) + k' y'_{1(0)}}{y_{1(0)}} . \tag{43}$$

The numerical value th

$$k_{12} = 27.8\ \mathrm{h}^{-1} .$$

Equation (26) is rearra

$$k_{e1} = \frac{-\lambda_1^2 - \lambda_1(k_{12} +$$
$$k_{12} + \lambda_1$$

Equation (26) is rearra

$$k_{21} = \frac{-\lambda_2^2 - \lambda_2(k_{12} +$$
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Equation (44) and (45)

$$k_{e1} = \frac{\lambda_1\lambda_2^2 - \lambda_1^2\lambda_2}{k_{12}(\lambda_2 - \lambda_1)}$$

and, with Eqs. (37) an

$$k_{e1} = \frac{\delta^2 k' - (k')^2\delta}{k_{12}(\delta - k')}$$

The numerical value th

$$k_{e1} = 2.03\ \mathrm{h}^{-1}$$ (whic

With Eqs. (37) and (45

$$k_{21} = \frac{(k')^2 - k'(k_{12} +$$
$$k'$$

and hence the numerica

$$k_{21} = 1.77\ \mathrm{h}^{-1} .$$

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The numerical value thus obtained is

$$k_{12} = 27.8\ h^{-1}. \quad (37)$$

Equation (26) is rearranged, and λ_1 (Eq. 27a) is inserted instead of λ:

$$k_{el} = \frac{-\lambda_1^2 - \lambda_1(k_{12} + k_{21})}{k_{12} + \lambda_1}. \quad (44)$$

Equation (26) is rearranged under insertion of λ_2 (Eq. 27b) instead of λ:

$$k_{21} = \frac{-\lambda_2^2 - \lambda_2(k_{12} + k_{el}) - k_{12}k_{el}}{\lambda_2}. \quad (45)$$

Equation (44) and (45) give

$$k_{el} = \frac{\lambda_1\lambda_2^2 - \lambda_1^2\lambda_2}{k_{12}(\lambda_2 - \lambda_1)} \quad (46)$$

and, with Eqs. (37) and (38),

$$k_{el} = \frac{\delta^2 k' - (k')^2\delta}{k_{12}(\delta - k')}. \quad (47)$$

The numerical value thus obtained is

$k_{el} = 2.03\ h^{-1}$ (which is the same as obtained above).

With Eqs. (37) and (45) we obtain

$$k_{21} = \frac{(k')^2 - k'(k_{12} + k_{el}) + k_{12}k_{el}}{k'} \quad (48)$$

and hence the numerical value

$$k_{21} = 1.77\ h^{-1}.$$

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