Environmental Sciences, 12, 6 (2005) 371–379
MYU Tokyo

ES605

Prediction of Systemic Concentrations of Sensitizing Compound Using TKTD Simulation Model

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Key words: TKTD model, simulation, dermal sensitization, mutagenicity, systemic concentration

To investigate the safe handling of an industrial product, *phenyl vinyl sulfone* (PVS), which has an extremely high potential for dermal sensitization at low concentrations and positive mutagenicity, the maximum no-effect concentration for dermal deposits was obtained from dermal sensitization experiments. The systemic concentrations in the liver, which is considered to be a target tissue of mutation, were monitored using the TKTD (Toxico Kinetics Toxico Dynamics) model by inputting the maximum no-effect concentration of sensitization. The predicted highest concentration in the liver was compared with the no-effect level of mutation in the same tissue, which was derived from an in vitro mutagenicity study. The results showed that when this product is handled at lower concentrations, which may not induce dermal sensitization, the systemic concentrations would be lower than those causing mutation in the liver. In workplaces, conditions that prevent dermal sensitization caused by PVS could also protect against the mutagenicity of this compound.

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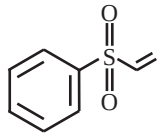
1. Introduction

There are numerous occasions in chemical companies when high production volumes of compounds have to be handled and few toxicity studies have been performed to confirm or evaluate the safety of workers and the establishment of safe workplace conditions. In fact, most chemicals are reactive. This reactive potential, in general, causes dermal irritation or dermal sensitization. In this study, we investigated the safe handling of a reactive compound causing dermal sensitization even at extremely low concentrations and which is also mutagenic. This compound is *phenyl vinyl sulfone* (PVS), an intermediate of chemical synthesis. First, the maximum no-effect concentration for dermal sensitization was obtained from animal experiments. In addition, the no-effect level in vivo in the liver was estimated by in vitro mutagenicity tests. Then, systemic exposure concentrations were monitored using the TKTD (Toxico Kinetics Toxico Dynamics) simulation model at a maximum no-effect concentration for dermal sensitization to compare with the no-effect level of mutation in the liver. At this exposure of the maximum no-effect concentration for dermal sensitization, it was evaluated that there would be low risks to the liver, which is considered to be the most probable target tissue in terms of mutation and dermal sensitization.

2. Materials and Method

2.1 *Compound*



PVS (Phenyl vinyl sulfone) CAS 5535-48-8

Table 1
Physical and chemical properties.

Shape	White powder
Molecular weight	168.21
Melting point	67–69°C
Vapor pressure	5 mmHg (160°C)
Kow	0.92 (log value)
Henry's law constant	–7.33 (log value)

2.2 *Maximization study*

The dermal sensitization study (maximization study) was conducted to obtain the no-effect concentration (dermal deposit concentration) of PVS using guinea pigs. This study was considered to be generally applicable to humans. The OECD guideline (1992)[1] and Magnusson and Kligman's Maximization study[2] were applied to the experiments.

Fifteen female Hartley guinea pigs of 300 g body weight supplied by Charles River Japan Inc. (Yokohama-city) were grouped into a no-treatment group (not exposed to PVS induced) of five and a treatment group (exposed to PVS) of ten.

First induction (intradermal injection):

A 1:1 emulsion of distilled water and FCA (Freund's Complete Adjuvant) was administered to the no-treatment group by intradermal injection. A 1:1 emulsion of 0.05% PVS in corn oil and FCA was administered to the treatment group by intradermal injection. The PVS concentration was derived from a preliminary study.

Second induction (topical):

Seven days after the first induction, a 5% PVS acetone solution (0.4 ml) on a filter paper (2×4 cm^2) was applied to the subjects in both groups for 48 h. The PVS concentration was derived from a preliminary study.

Challenge (topical):

Two weeks after the second induction, an acetone solution (0.01 ml) of 0.1%, 0.01%, 0.001%, 0.0001% or 0.00001% PVS was patched to the left flanks of the animals.

Observation:

Dermal reactions (erythema and edema) were observed at 24 h, 48 h and 72 h after the patch application.[3]

2.3 *Mutagenicity study*

The mutagenic potential of PVS was determined by bacterial reverse mutation assays using four strains of *Salmonella typhimurium* (TA100, TA98, TA1535 and TA1537) and *Escherichia coli* (WP2uvrA) in the presence or absence of a rat liver drug-metabolizing enzyme system (S9mix). Following the preincubation step, the reverse mutation assays were performed. PVS was dissolved in DMSO (dimethyl sulfoxide) to give concentrations of 4.88, 9.77, 19.5, 39.1, 78.1, 156, 313, 625 and 1250 μg/plate in 0.1 ml. The PVS DMSO solution (0.1 ml), either Na-phosphate buffer (pH7.4, 0.5 ml) or S9 mix (0.5 ml), and the bacterial suspension (0.1 ml) were mixed and incubated at 37°C for 20 min. Soft agar (2 ml) was added to each plate and plates were incubated at 37°C for 48 h. The plates were then stored at 4°C until colony counting.[4]

2.4 *TKTD simulation model*

The simulation model used here to predict systemic concentration was constructed following the Andersen and Clewell's approach[5] (Fig. 1) with a slight modification.[6] Using the model, liver and vein PVS concentrations were monitored at the maximum no-effect concentration for dermal sensitization estimated from the Maximization study.

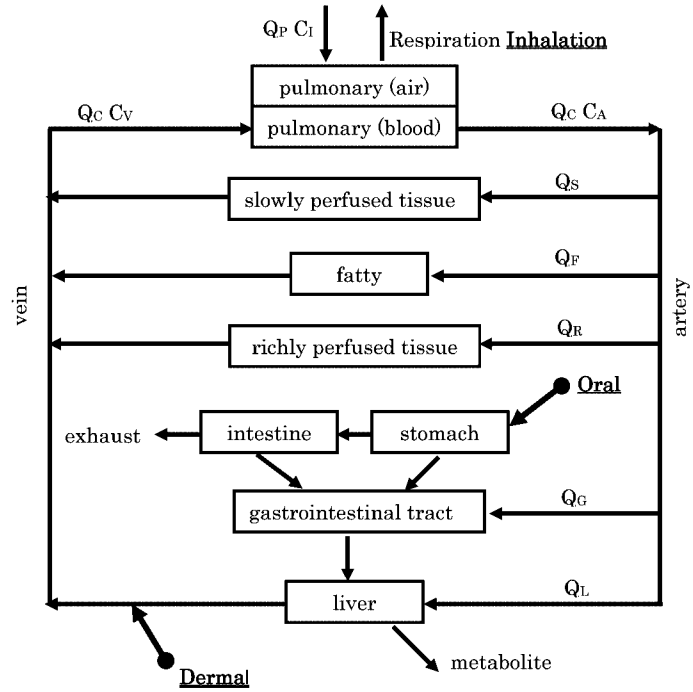


Fig. 1. Diagram of physiologically-based pharmacokinetic model. The abbreviations are as follows: C_V, C_A, C_I: concentrations of chemical in each tissue (mg/L), Q_P: pulmonary ventilation (L/h), Q_C, Q_S, Q_R, Q_F, Q_G, Q_L: blood flows in each tissue (L/h).

The differential equations relating to the mass balance of a chemical are as follows: (For details, see Appendix A)

Liver mass balance:

$$dC_{L(liver)}/dt = (Q_{L(liver)} \times (C_{A(artery)} - C_{L(liver)} / P_{L(liver)}) / V_{L(liver)} + (Q_{G(G.I.tract)} \times (C_{G(G.I.tract)} / P_{G(G.I.tract)} - C_{L(liver)} / P_{L(liver)}) / V_{L(liver)} + K \times C_{L(liver)} / P_{L(liver)} + V_{max} \times C_{L(liver)} / P_{L(liver)} / (K_m + C_{L(liver)} / P_{L(liver)})$$

Whole body mass balance:

$$C_{V(vein)} = (Q_{S(slow)} \times C_{S(slow)} / P_{S(slow)} + Q_{R(rich)} \times C_{R(rich)} / P_{R(rich)} + Q_{F(fat)} \times C_{F(fat)} / P_{F(fat)} + (Q_{G(G.I.tract)} + Q_{L(liver)}) \times C_{L(liver)} / P_{L(liver)}) / Q_{C(blood)} + C_{DEM(dermal)}$$

Abbreviations:

$C_{V(vein)}$, $C_{S(slow)}$, $C_{F(fat)}$, $C_{R(rich)}$, $C_{G(G.I.tract)}$, $C_{L(liver)}$: concentrations of chemical in each tissue (mg/L)

$C_{DEM(dermal)}$: exposure concentration via dermal route (mg/L)

$Q_{C(blood)}$, $Q_{S(slow)}$, $Q_{F(fat)}$, $Q_{R(rich)}$, $Q_{G(G.I.tract)}$, $Q_{L(liver)}$: blood flows in each tissue (L/h)

$P_{S(slow)}$, $P_{F(fat)}$, $P_{R(rich)}$, $P_{G(G.I.tract)}$, $P_{L(liver)}$: each tissue/blood partition coefficient

$V_{L(liver)}$: volume (weight) of each tissue (kg)
V_{max}: maximum velocity
K_m: Michaelis-Menten constant
K: clearance
t: time

3. Results and Discussion

3.1 *Maximization study*

The dermal reactions of erythema and edema were classified and the number of animals that reacted was counted (Table 2).

The challenge using the five concentration levels revealed 0.0001% as the maximum no-effect concentration.

It is unusual to determine the no-effect concentration of dermal sensitizing products. However, it has been reported in the literature that there is a threshold value for induction or challenge levels and that the threshold challenge value could be obtained. Even if sensitization has already been induced, when the exposure level is lower than the threshold challenge value, no allergic reactions are observed.

It was also reported that the induction and challenge concentrations are very similar,[7] and it can be considered that a low concentration with no effects will not induce T-cell sensitization. In any case, our results indicate the necessity of considering the safe handling of substances, which have a high potential to cause sensitization.

3.2 *Mutagenicity study*

The mean value of colony counts for two plates is shown in Table 3. The result for *Escherichia coli* (WP2uvrA) showed a clear dose-response curve and the assay reacted from the lowest dose, which is considered to be the lowest limit of this assay. The no-effect levels of mutation were estimated from this dose-response curve to be 1.22 μg/plate (without S9mix) and 4.88 μg/plate (with S9mix). The no-effect concentrations were calculated as follows:

$$\begin{aligned} 1.22\ \mu g/plate &= 1.22\ \mu g/(2\ ml+0.5\ ml+0.5\ ml+0.1\ ml+0.1\ ml) \\ &= 0.38\ \mu g/ml = 0.38\ mg/L \\ 4.88\ \mu g/plate &= 1.53\ mg/L \end{aligned}$$

Table 2
Results of maximization study in guinea pigs.

Concentration	Sensitization ratio
0.1% (1000 ppm)	10/10 (100%)
0.01% (100 ppm)	10/10 (100%)
0.001% (10 ppm)	1/10 (10%)
0.0001% (1 ppm)	0/10 (0%) ← maximum no-effect concentration
0.00001% (0.1 ppm)	0/10 (0%)

Table 3
Results of mutagenicity study with *E.coli* (mean number of colonies/2 plates).

Dose (μg/plate)	WP2uvrA (without S9mix)	WP2uvrA (with S9mix)
0	23	25
1.22	35	—
2.44	63	—
4.88	104	22
9.77	177	49
19.5	307	165
39.1	531	381
78.1	—	693
156	—	882

—: Not tested

In mutagenicity studies, we usually evaluate chemicals as being positive or negative, or qualitatively as strong or weak. Quantitative evaluation is used, for example, when studying a possible relationship to carcinogenicity potential. The approach adopted in this study, that is, determining the no-effect concentration for mutagenicity is rather unique. However, from the viewpoint of risk assessment, the no-effect concentration is as valuable as the other common endpoints. PVS seems to react directly in a nucleophilic manner with a DNA base (H-Base) (Fig. 2 First-scheme).

However, mutagenic activity was decreased with the addition of S9 Mix and thus, PVS may first react in a nucleophilic manner with epoxidase to yield an intermediate epoxide, which then reacts with H-Base (Fig. 2 Second-scheme). The target tissue of this type of mutagenicity is considered to be the liver, because the liver contains this enzyme abundantly. Thus, the systemic levels of PVS were calculated in the liver.

3.3 *Prediction of systemic concentration using TKTD model*

The systemic concentrations in the liver and vein were calculated using the TKTD simulation model for the 6 h of dermal exposure for male humans at a PVS concentration of 1ppm, which corresponds to the maximum no-effect level in terms of sensitization.

The parameter values are shown in Appendix A. The dermal exposure area of guinea pigs (2×4 cm^2) was extrapolated from that of humans (8810 cm^2: face, hands and arms).[8] The concentrations reached maxima at 6 h of exposure and were 0.351 mg/L in the liver and 0.538 mg/L in the vein(Fig. 3). These values are lower than the no-effect concentrations for mutagenicity (without S9mix, 1.22 μg/plate = 0.38mg/L; with S9mix, 4.88 μg/plate = 1.53 mg/L); they were 1/1,083; 1/4,361 in the liver and 1/706; 1/2,841 in the vein.

In addition, in this study it was assumed that the sensitivities of liver cells and *Escherichia coli* to PVS were almost the same. This product is an intermediate used in the synthesis of chemicals. Workplace exposure may involve only skin penetration. Thus, we concluded that if the workplace exposure level can be kept under the maximum no-effect concentration for dermal sensitization, mutagenicity in vivo can be prevented and PVS can be used on an industrial scale.

This simulation model uses the US EPA theory of dermal penetrating factor based on the

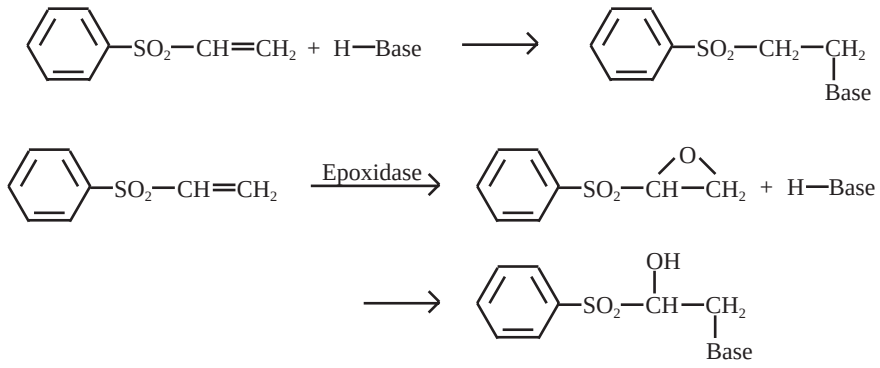


Fig. 2. Reaction schemes of PVS with base of DNA.

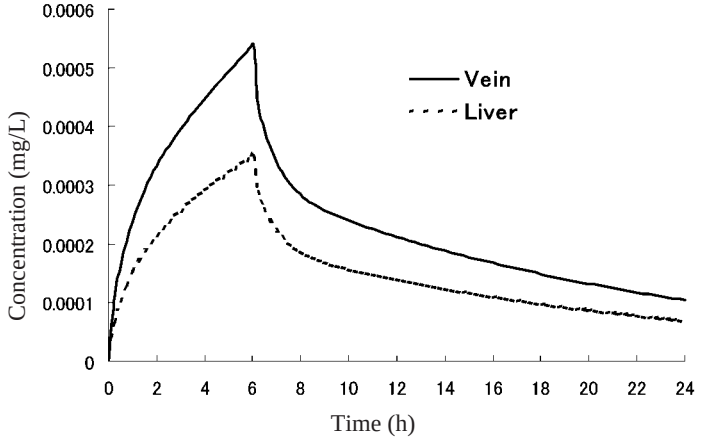


Fig. 3. Concentrations (mg/L) of PVS in liver and vein following dermal exposure for 6 h. (Human)

Quantitative Structure-Activity Relationship in predicting the systemic concentration by dermal exposure.[9] That is, penetrating factor (Kp) is a function of logP as shown in Appendix A.

$$\log Kp = 0.71*\log P - 0.0061*MW - 2.72 \ (n = 212, R = 0.98, SE = 0.220)$$

The chemical domain for this equation has molecular weights ranging from 18 to 697 and logP values ranging from –2.1 to 6.8. PVS is included in these ranges and is thought to be applicable.

4. Conclusion

To establish the safe handling of the industrial chemical PVS, which has an extremely high potential for dermal sensitization at low concentrations and positive mutagenicity, the maximum no-effect concentration for skin deposits was estimated from dermal sensitization experiments. A mutagenicity study was also performed to determine the no-effect level for mutagenicity. By using the maximum no-effect concentration for dermal sensitization, the systemic level in the liver, which is considered to be a target tissue, was predicted using the TKTD model and compared with the no-effect level for in vitro mutation. The results showed that when PVS was used at low concentrations, which did not induce dermal sensitization, the systemic concentrations ranged from 1/4300 to 1/700 which were lower than those of the no-effect level for mutagenicity in the liver. Thus, it is concluded that there are no concerns regarding dermal sensitization and mutagenicity if this product is handled with care by maintaining occupational skin exposure levels lower than the maximum no-effect concentration for dermal sensitization.

Acknowledgements

The study was supported partly by the Program of the Japan Chemical Industry Association. The authors thank Dr. Tsutomu Nishihara of Osaka University for his encouragement.

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