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7 VCM Literature

April 26, 1977

TO: Vinyl Chloride Technical Panel

SUBJECT: A Statistical Assessment of the Quantitative Uptake of Vinyl Chloride Monomer from Aqueous Solution

Gentlemen:

Because of its relevancy to research currently in progress the attached subject paper is being made available to all Panel members.

J. T. Seawell

J. T. Seawell
Project Manager
Vinyl Chloride Research

JTS:cc
Attachment

A STATISTICAL ASSESSMENT OF THE QUANTITATIVE UPTAKE OF VINYL CHLORIDE MONOMER FROM AQUEOUS SOLUTION

Jim R. Withey

Foods Directorate, Bureau of Chemical Safety,
Toxicology Division, Ottawa, Canada

Brian T. Collins

Foods Directorate, Division of Food Statistics and
Operational Planning, Ottawa, Canada

The presence of vinyl chloride monomer (VCM) in foodstuffs and its demonstrated carcinogenic potential when administered by the oral route has raised questions concerning the quantitative estimation of the safety of the use of food packaging fabricated from rigid polyvinyl chloride. A statistical model, which was tested by curve-fitting data obtained from an oral uptake study, has been demonstrated to be of predictive value. Ninety-five percent confidence limits were also calculated, and the data from this study were compared with those from a previous gas phase exposure study. It was concluded that if the total daily liquid intake contained 20 ppm of VCM then the area generated under the blood level-time curve, for rats, would be equivalent to an inhalation exposure of about 2 ppm for 24 hr.

INTRODUCTION

Recent work in our own laboratories and elsewhere (Fuchs et al., 1975; Williams and Miles, 1975) has demonstrated the presence of VCM in foodstuffs. Pharmacokinetic and uptake studies (Withey, 1976) illustrated that uptake from aqueous and lipid solutions containing VCM was extremely rapid and complicated. Since the variability of the uptake of VCM in aqueous solution from the GI tract did not allow a complete pharmacokinetic analysis of observed blood level data, it appeared that a quantitative measure of uptake was possible only from a statistical analysis of the area under the blood level-time curves (AUC), which could be obtained after dosing. A similar approach has been frequently used for the assessment of the bioavailability of oral drug dosage forms (Wagner, 1971).

It is a pleasure to acknowledge the skill and dedication of Peter Collins, who assisted with the analytical work, and of Henry James, who surgically prepared the animals that were used in this study.

Requests for reprints should be sent to Jim R. Withey, Foods Directorate, Bureau of Chemical Safety, Toxicology Division, Tunney's Pasture, Ottawa K1A 0L2 Canada.

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After an examination of data from a pilot study of the uptake of VCM from aqueous solution when administered intragastrically, an experimental design was conceived in which uptake was assessed for rats of two different body weight groups at five different dose levels for each. The dose levels ranged from 2 to 25 mg, the lower dose level being limited by the analytical sensitivity for VCM in blood and the upper dose level by the solubility of VCM in water. The dose-reponse curve data, in terms of the AUC against dose, was then curve-fitted to two model equations and statistically analyzed. The models were studied for goodness of fit to the data and both were found to provide an adequate fit for the data obtained from the 200-g animals. The two models were then extrapolated to predict the uptake for aqueous solutions of VCM of lower concentrations than those used in the experiment. Results of this study were compared with those from a previous study in which uptake of VCM from gas phase exposures was measured.

METHODS AND EXPERIMENTAL DESIGN

Sixty male Sprague-Dawley rats, equally divided into two weight groups of approximately 200 and 400 g, were surgically prepared with an indwelling cannula 48 hr prior to dosing. Six animals from each weight group were dosed intragastrically by means of a flexible cannula at each of five dose levels of 2, 4, 8, 16, and 25 mg of VCM, contained in 5 ml of water. A simple factorial dosing experiment was selected so as to allow the effects of animal body weight, dose, and their interaction to be assessed. The order of dosing was then derived by randomly assigning doses and animal weight combinations with the restriction that two animals could be dosed daily. The animals were deprived of food and water 16 hr prior to dosing since these were known, from preliminary studies, to perturb the rate and extent of VCM uptake. Immediately after the removal of food and water, each animal was dosed by stomach intubation, with 5 ml of water so as to reduce desiccation and excessive overnight weight loss. Animals were weighed prior to the deprivation of food and again prior to dosing.

Aqueous solutions of VCM were prepared and analyzed just prior to dosing by methods which have been described previously (Withey, 1976). Blood samples (0.1 ml) were taken at 2, 4, 8, 12, 16, and 20 min after dosing and then every 10 min up to 2 hr or until levels of VCM were undetectable.

RESULTS

The area under each blood level-time curve was calculated, using the trapezoidal rule, from the equation

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$$\mathrm{AUC} = \sum_{i=0}^{n} \frac{(t_{i+1} - t_i)\,(C_{i+1} + C_i)}{2} \qquad (1)$$

where C_i was the concentration of VCM in the blood at time t_i and there were n observations. It was assumed that the blood concentration was zero at time $t_0 = 0$.

The AUC for each animal, the actual dose administered, and the weight of the animal just prior to dosing are given in Table 1a and b.

TABLE 1. Actual Body Weights of Animals, Administered Dose, and Area under Curve

Required dose (mg)	BW, AD, and AUC[a]	1	2	3	4	5	6	Mean	SD	CV
		(a) Light group (~ 200-g body weight)								
	BW	205	234	191	223	215	190	209.7	17.64	0.08
2	AD	2.28	2.28	1.95	2.31	2.23	2.20	2.21	0.133	0.06
	AUC	63.90	40.34	38.81	25.76	50.24	65.11	44.36	15.39	0.33
	BW	214	210	198	235	225	219	216.8	12.73	0.06
4	AD	4.21	4.11	4.27	4.31	4.11	4.11	4.19	0.090	0.02
	AUC	75.89	76.18	90.16	108.6	68.45	102.0	86.87	16.04	0.185
	BW	193	209	228	235	213	222	216.7	15.00	0.07
8	AD	7.96	7.55	7.64	7.64	7.87	8.31	7.82	0.283	0.04
	AUC	137.3	355.8	322.1	260.1	112.3	203.9	231.9	98.30	0.42
	BW	204	222	213	217	204	222	213.6	8.21	0.04
16	AD	16.66	16.05	16.02	16.84	15.36	16.23	16.19	0.526	0.03
	AUC	403.1	327.5	227.0	611.5	308.7	293.9	361.9	134.8	0.37
	BW	205	202	215	191	200	226	206.5	12.31	0.06
25	AD	25.26	25.84	25.99	25.53	24.93	24.24	25.30	0.645	0.03
	AUC	654.7	610.2	696.7	840.5	596.5	525.4	654.0	108.0	0.16
		(b) Heavy group (~ 400-g body weight)								
	BW	405	398	387	354	381	364	381.5	19.58	0.05
2	AD	2.18	2.18	1.99	1.95	2.31	2.23	2.14	0.141	0.07
	AUC	15.14	23.75	10.56	18.81	14.40	17.55	16.7	4.47	0.27
	BW	387	352	381	368	372	381	373.5	12.57	0.03
4	AD	4.21	4.43	4.31	4.25	4.25	4.01	4.24	0.138	0.03
	AUC	53.56	37.35	29.64	41.14	49.11	48.92	43.29	8.912	0.21
	BW	422	427	333	363	351	384	380.0	38.28	0.10
8	AD	8.08	7.88	8.36	8.15	8.15	8.34	8.16	0.177	0.02
	AUC	124.7	114.9	392.8	131.2	95.92	107.9	161.2	114.1	0.71
	BW	390	351	360	340	355	358	359.0	16.76	0.05
16	AD	16.35	15.36	16.14	16.23	16.78	15.39	16.04	0.561	0.03
	AUC	295.6	300.2	469.9	215.4	370.0	219.3	311.7	96.57	0.31
	BW	383	380	377	353	363	377	372.2	11.63	0.03
25	AD	24.34	25.42	23.88	24.75	24.64	24.64	24.61	0.507	0.02
	AUC	511.2	432.2	394.2	393.7	380.6	519.4	438.6	61.95	0.14

[a] BW is the actual body weight (g); AD is the actual dose administered; AUC is the measured area under the blood level-time curve (min-μg/ml); CV is the coefficient of variation.

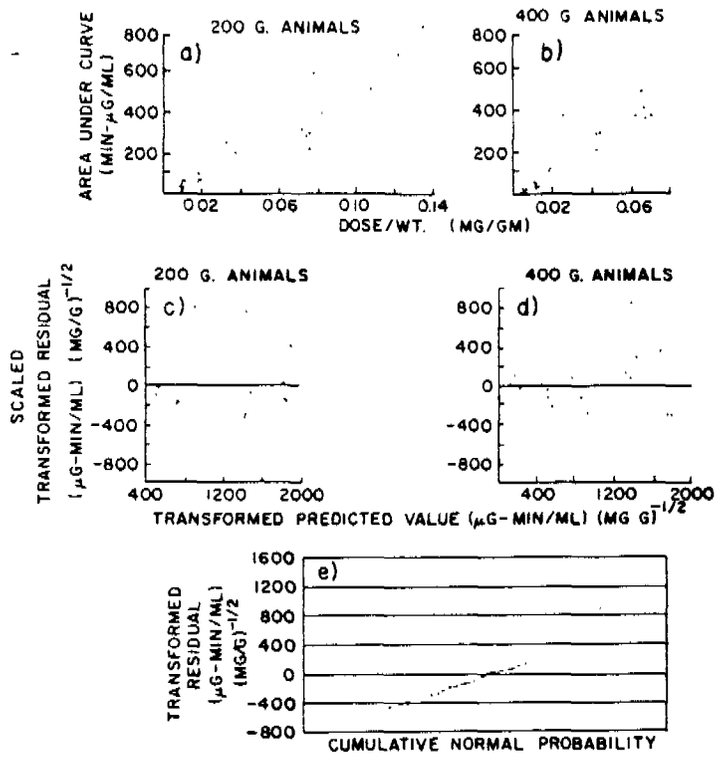

FIGURE 1. Curve-fitting analysis for model I. (a) and (b) Area under curve against dose for 200-g and 400-g animals. (c) and (d) Scaled transformed residuals against transformed predicted values of area under curve. (e) Cumulative normal probability plot of the transformed scaled residuals for all animals.

ANALYSIS OF DATA

Plots of the AUC against the actual administered dose, in mg/kg, were made for the two animal weight classes used in the experiment and are presented in Fig. 1 a and b. A weighted regression was then performed for the sets of data according to the model equations (model I) below:

$$(\text{AUC})_i = \alpha_0 + \alpha_1 \, (D/W)_i + \epsilon_i \quad \text{for animals weighing about 200 g} \quad (2a)$$

and

$$(\text{AUC})_i = \beta_0 + \beta_1 \, (D/W)_i + \epsilon_i \quad \text{for animals weighing about 400 g} \quad (2b)$$

where $(\text{AUC})_i$ was the calculated area under the blood level-time curve for animal i in the 200-g or 400-g weight class. The α's and β's were unknown coefficients that were required to be estimated, $(D/W)_i$ was the actual dose

in mg divided by the weight of the animal in grams for animal i, and ϵ_i was the random error associated with each animal. The values of ϵ_i were assumed to have an average of zero and a variance equal to $\sigma^2 (D/W)_i$, where σ^2 is an unknown parameter common to both weight classes.

After the regression was performed the residuals were transformed (Draper and Smith, 1966) because a weighted regression was performed. The residuals were also scaled to have equal variances (Timm and Carleson, 1974). A plot of transformed scaled residuals against predicted values of AUC for each animal weight class and a cumulative normal probability plot of the transformed scaled residuals (Daniel and Wood, 1971) were made.

It was apparent from an inspection of these graphs that one point in the 400-g weight class was an outlier. This observation was obtained for animal 3, which had received a dose of 8.36 mg but which had yielded an AUC more consistent with those that received a 16-mg dose. This observation was therefore rejected and the analysis was repeated. No further outliers were observed after the second regression.

An F test and a Scheffe's F projection test (Miller, 1966) on the AUC and (D/W) data showed that the data may pass through the origin for the 200-g weight class but that they did not for the 400-g weight class. Since it was known that the relationship must pass through the origin it was concluded that either the model or the observed data must be in error for the 400-g weight class. Since the AUC was assumed to be zero after VCM blood levels became undetectable the observed data may be biased low. Thus there may be a significant contribution to the values of AUC from the terminal phase of the blood level-time curve especially at low values of (D/W).

The coefficients, estimated after setting $\alpha_0 = 0$, are shown in Table 2. This regression allowed a reduction in the sum of squares (r^2) of 76%. The plots of scaled transformed residuals against transformed predicted value and a cumulative normal probability plot of the scaled residuals are shown in Fig. 1, c, d, and e. An approximately equal number of points lie above and below the baseline in Fig. 1, c and d, as would be expected if the model is correct. From Fig. 1d it was observed that for the 400-g class the variance appeared to increase with predicted value, again indicating that the model may not be appropriate.

TABLE 2. Coefficients for the Fitted Eqs. (2a) and (2b), Model 1, After Setting $\alpha_0 = 0$ and Elimination of the Outlier

Coefficient	Estimated value	SE
α_1	5215	246.8
β_0	27.55	9.370
β_1	7145	456.0

Since model I appeared not to pass through the origin for the 400-g weight class, alternative regressions were examined. After some preliminary plots of the data were made, the model (model II) given in Eq. (3) was chosen for further study and a least-squares regression was applied to the data.

$$\log(\text{AUC})_j = \gamma_0 + \gamma_1 (\log \text{weight})_j + \gamma_2 (\log \text{dose})_j + \gamma_3 (\log \text{weight})_j (\log \text{dose})_j + \delta_j \quad (3)$$

where $\log(\text{AUC})_j$ was $\log_{10}$ of the measured AUC for animal j, $(\log \text{weight})_j$ was $\log_{10}$ of the actual body weight for animal j, $(\log \text{dose})_j$ was $\log_{10}$ of the actual dose administered to animal j, the γ's were coefficients to be estimated, and δ_j was a random deviation associated with the jth animal. The values of δ_j were assumed to follow a normal distribution with an average of zero, a variance of σ^2, and all δ's were assumed independent.

The residuals were scaled to have equal variance, and a plot of scaled residuals against predicted value, a cumulative normal probability plot, and plots of partial residuals (Larsen and McCleary, 1972) were made. An assessment of these graphs was made and it was decided that animal 3 in the 400-g class was an outlier for this model as well as for model I. The coefficients γ from model II were reestimated after exclusion of the outlier and are shown in Table 3. This regression allowed a reduction in the sum of squares (r^2) of 94%. Revised plots of scaled residuals against predicted value, partial residual plots, and a revised cumulative normal probability plot were made, as shown in Fig. 2, a, b, c, d, and e, respectively.

An F test of the significance of including the coefficient α_3 in the model was also performed (Draper and Smith, 1966). The hypothesis that $\alpha_3 = 0$ was rejected ($p < 0.05$), indicating that α_3 was a necessary term in the model.

Confidence regions were calculated for the regression surfaces of models I and II (Miller, 1966). Figure 3 illustrates plots of the AUC against dose, in mg, after rejection of the outlier together with their estimated confidence bands for animals weighing exactly 200 and 400 g.

TABLE 3. Coefficients for the Fitted Eq. (3), Model II

Coefficient	Calculated value	SE
γ_0	5.954	0.7688
γ_1	−2.006	0.3134
γ_2	−1.643	0.7964
γ_3	1.169	0.3251

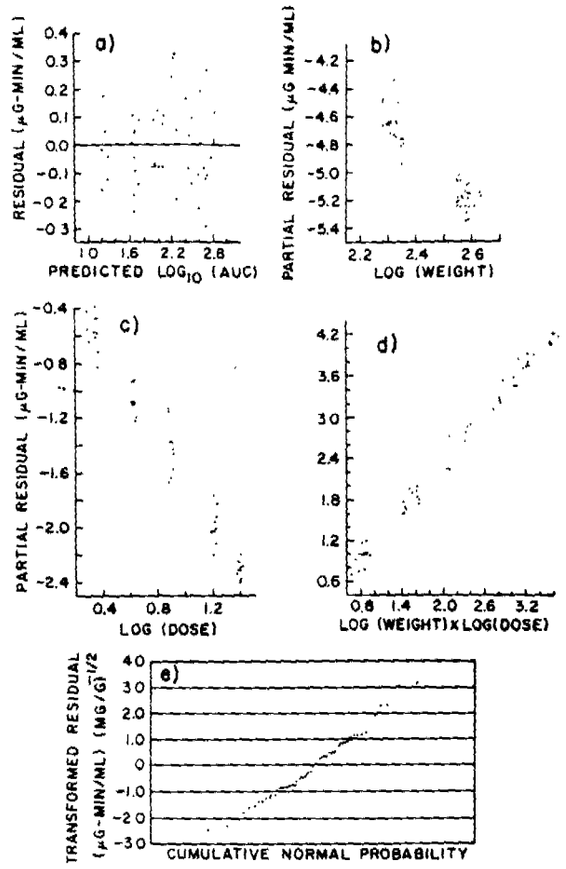

FIGURE 2. Curve-fitting analysis for model II (after exclusion of the outlier). (a) Scaled residuals against predicted $\log_{10}$(AUC). (b), (c), and (d) Partial residuals against $\log_{10}$(weight), $\log_{10}$(dose), and $\log_{10}$(weight) X $\log_{10}$(dose). (e) Cumulative normal probability plot.

The lines in Fig. 3, a and b, were calculated from Eqs. (4) and (5) derived from model I.

For 200-g animals

$$\text{AUC} = 26.08D \tag{4}$$

and for 400-g animals

$$\text{AUC} = 27.55 + 17.86D \tag{5}$$

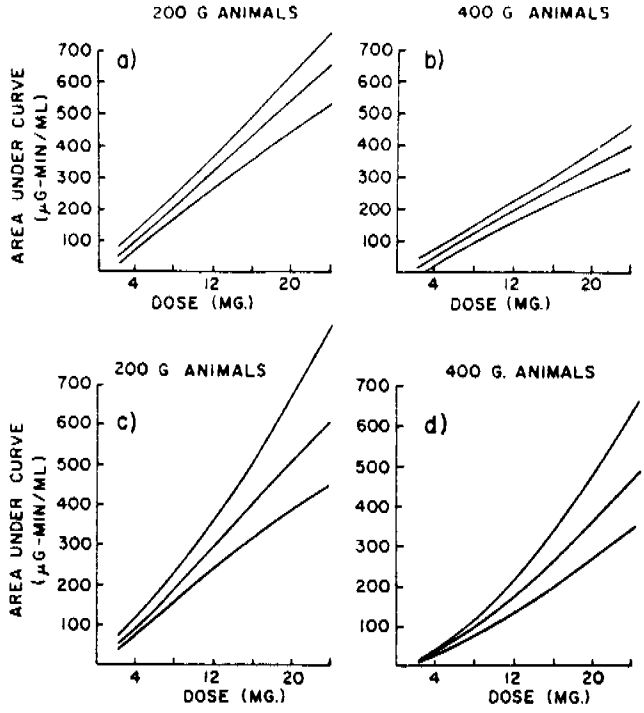

FIGURE 3. Area under curve against dose with estimated confidence bands. Parts (a) and (b) are for model I, (c) and (d) for model II.

Figure 3, c and d, were calculated from model II, in which the derived equations were as follows:

For a 200-g animal

$$\log \text{AUC} = 1.3378 + 1.0458 \log(\text{dose}) \quad (6)$$

and for 400-g animal

$$\log \text{AUC} = 0.7339 + 1.3977 \log(\text{dose}) \quad (7)$$

DISCUSSION

The expected linear relationship between the administered dose, on a dose-weight basis, and the evoked response, in terms of the generated AUC (i.e., model I), appeared to be followed reasonably well by the data for the 200-g weight class. For the 400-g class, however, the regression equation did not pass through the origin and inspection of the scaled

transformed residual plots showed some inadequacies in the fit of the data.

Since model I was inadequate to fit the data for the 400-g class, an alternate model (model II) was examined and the data appeared to fit model II very well. The plots of scaled residuals against predicted value, scaled residuals against cumulative normal probability, and the partial probability plots were all observed to appear as expected. The linearity of the relationship between log AUC and the factors log(dose) and log(weight) X log(dose) was visually confirmed by inspection of the partial residual plots. However, since only two weight classes were observed it was not possible to ascertain whether a linear relationship existed between log(AUC) and weight. Hence, even although it was possible to interpolate or extrapolate model II to weights other than 200 or 400 g, it was not considered reliable to do so. A precise relationship of AUC to animal body weight for a given dose may be difficult to establish owing to the factors which affect the elimination of VCM from the blood compartment. It has been shown (Schaumann, 1934) that more than 82% of VCM that is absorbed into the blood compartment is excreted by way of the pulmonary route, and lung tidal volumes with body weights, for rats, has been reported to vary as the 0.67 power of the body weight (Leong et al., 1964). The tidal volume and breathing rate would influence the elimination rate for VCM, and the latter has been shown to influence the AUC where first-order kinetic processes are involved (Wagner, 1971).

The carcinogenic potential of VCM has now been demonstrated at very low levels of chronic gas phase exposure. Moreover, the variety of tumors that can now be associated with VCM exposure (Maltoni and Lefemine, 1974; Thomas et al., 1975; Viola et al., 1971) has established that tumors have appeared in any organ that is provided with a good blood supply. Thus the VCM blood concentration-time curve for an animal may be considered as a useful toxicological parameter in assessing the carcinogenic risk arising from the uptake of VCM from any route of ingestion.

Early findings by the U.S. Food and Drug Administration (*Fed. Regist.*, 1975) of up to 20 ppm of VCM in alcoholic beverages, led to the withdrawal of the prior sanctioned use of rigid polyvinyl chloride as a food-packaging material. More extensive analysis of foods packaged in this material (van Esch and van Logten, 1975; Williams and Miles, 1975) revealed the presence of VCM at up to 10 ppm of VCM, although manufacturers now claim to have reduced the VCM content of the rigid plastic (*Food Chem. News*, 1975).

It has been reported that a rat of 200-g body weight consumes about 45 ml of water per day, so that if this total liquid intake contained 20 ppm of VCM, then the ingested dose would be 0.9 mg of VCM. When the dose response curves expressed as Eqs. (4) and (6) were extrapolated to a dose of 0.9 mg the predicted AUC's were 23.5 and 19.5 min-μg/ml, respectively. While it is appreciated that extrapolation of experimental

results must always be used with reservation, this extrapolation is not very far beyond the lower limit of the experimental dose range (2–25 mg) and the dose–response equation appeared to fit reasonably well over the entire experimental range for the animals of 200-g body weight. Since the two predicted values were quite similar either may be used in the following discussion; for example, let the predicted AUC of 19.5 min-μg/ml be used.

It has been demonstrated (Withey, 1976) that when a rat of any weight was exposed to a constant concentration of VCM in the gas phase, the blood compartment rapidly equilibrated to give a plateau or equilibrium blood concentration of VCM which was directly proportional to the exposure concentration. This relationship was found to obey the linear equation $y = 0.3832x$, where y was the exposure concentration in μg/ml and x was the equilibrium blood concentration of VCM in μg/ml. This relationship was found to be independent of the body weight. Thus a blood equilibrium concentration of 19.50 μg/ml would be predicted for a rat exposed to a constant atmospheric concentration of VCM of 7.471 μg/ml, which is equivalent to 2834 ppm. If the AUC can be considered as a parameter that is directly proportional to the toxicological effect (i.e., the induction of tumors), then it should be possible to equate equivalent values of AUC no matter whether these were generated as a consequence of an exposure to VCM in the gas phase or after the administration of an intragastric dose. In the case of VCM an AUC of 19.50 min-μg/ml would be generated in an atmosphere of 2834 ppm for 1 min or 1.97 ppm for 24 hr, and this would represent an exposure hazard equivalent to the ingestion of 0.9 mg of VCM by a 200-g rat. These conclusions were based on a comparison of two fitted equations and should therefore be considered as only approximate; however, it would appear that the hazards presented by the presence of VCM in foodstuffs at concentrations near 20 ppm could exceed those in the industrial work place for which limits have now been proposed at below 1 ppm per 8-hr day (Anonymous, 1974).

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