Environmental Health Perspectives
Vol. 42, pp. 9-13, 1981

Dose-Response Analysis in Animal Studies: Prediction of Human Responses

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An animal bioassay has been used to evaluate a series of airborne chemicals for their sensory irritating properties to the upper respiratory tract. The results obtained can be used to rank their potency. An attempt has been made to predict "safe" levels of exposure for humans on the basis of this short-term assay. A good correlation was obtained between the predicted "safe" levels of exposure and current Threshold Limit Values established for industrial exposures.

Introduction

Epidemiologists and toxicologists have shared a concern about the effects of chemicals on human health. Both are involved in devising protocols to study the effects of chemicals in order to arrive at "safe" level of exposure. Both face the same difficulties in studying low levels of exposure and low risk situations. While toxicologists must face the dangerous business of extrapolation from animals studies to man, the epidemiologist is never certain about exposure levels and the many variables which may have been overlooked. Despite such difficulties a variety of models for data analysis have been proposed and used in toxicological as well as epidemiological studies (*1,2*). Assuming for a moment that such models permit us to extrapolate from toxicological data obtained in long-term chronic studies (*2*) within reason—and it looks that way—the long-term chronic study remains to be done. The question is whether there is any possibility to use short-term tests reliably to predict and evaluate chemicals prior to undertaking such studies. During the past 10 years, there has been a great emphasis on such short-term tests but it seems that the emphasis has been more on qualitative than on quantitative predictions, and none of them are applicable to inhalation exposure. I would like to present a somewhat different approach which, although empirical, is nonetheless no more so than the extrapolation models so popular now (*1*).

Thirty years ago, the British pharmacologist Frazer (*3*) had the same concern about chemicals as we have today, primarily with respect to food additives, the main concerns of that time period. The same questions were raised then about long-term toxic effects of chemicals. Reflecting on his experiences, he proposed that some extrapolations could be made from acute studies if one obtained a dose-response relationship for a major biological effect of the chemical in question. Then extrapolation could be made from the 50% effect level by taking various ratio of the dose producing this level of effect. Using this scheme, we can propose the relationship given in Table 1. Note that there are not only two variables to consider—dose and response—usually discussed on toxic effects, but a third variable, duration of administration, has been entered. This general approach has been used by many toxicologists in selecting dose level for long-term chronic studies in animals. However, the real question is whether such an approach can be used whereby the same ratio would be used to predict, from the acute studies in animals, what would be a "safe" level for humans.

Obviously an "appropriate" animal model must be available whereby the biological effect observed can be qualitatively correlated with a particular effect in man. It is not necessary, however, that

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Table 1. A proposed approach combining quantity, quality of the effect and duration of administration required of an airborne contaminant.

Quantity	Types of effects	Duration of exposure required or permitted
10	Lethal	Minutes
1	Toxic: tissue damage	Hours
0.1	Effective: pharmacological reaction	Hours-days
0.01	Ineffective: within physiological limits	Weeks-years
0.001	Completely safe	Years, continuously

the effect observed in animal be the same as that in humans, it is only required that one predicts the other.

A dose-response curve must be obtained. Since the midpoint will be used for extrapolation it is not necessary to carry on a large number of experiments nor are sophisticated statistical approaches required. A least-squares linear regression analysis method is all that is needed.

A literature search must be conducted for the effect of the chemicals in humans. Obviously epidemiological studies of human exposed at very low levels must be avoided at first. One must try to find exposure levels within the range tested in the acute animal model and then proceed to the other ranges and longer duration of exposure. Too often we get carried away with prediction of "safe" levels for long-term exposures, and we forget that we have many episodes of short-term exposures which have yielded more reliable data in terms of exposure conditions as well as measurement of the effects.

Attempt to Verify the Above Approach

For a series of airborne chemicals having sensory irritating properties, we attempted to verify how appropriate the approach presented in Table 1 would be. I do not want you to think that a systematic approach can be followed from the beginning. Indeed there were many "best guesses" involved and it took many years to arrive at some useful results. In particular, much discussion was conducted with Dr. Henry F. Smyth, Jr. and his first-hand knowledge and experience in establishing Threshold Limit Values (TLVs) for industrial exposure to airborne chemicals was invaluable.

Type of Chemicals Selected

One basis for establishing a TLV is quite simply to prevent complaints of eye, nose and throat irritation in workers, commonly referred to as "sensory irritation." Indeed, an evaluation done by H. F. Smyth, Jr. (personal communication) showed that this was the primary basis for the TLV for about 40% of the industrial chemicals for which we have a TLV. His evaluation also showed that at levels three to four times the TLV, 66% of the chemicals listed would elicit sensory irritation. Obviously, they can elicit a wide variety of other toxic effects, but it seems that if the exposure level is low enough to prevent sensory irritation these other toxic effects are unlikely to occur. Therefore, a series of these chemicals was tested in an animal model to determine their potency as sensory irritant and prediction of human responses developed following the approach given in Table 1.

Animal Model and Qualitative Correlation

The animal model was devised in 1966 (*4*). Briefly, its basis is as follows. When airborne chemicals impinge on the nasal mucosa, the trigeminal nerve endings are stimulated, and inhibition of respiration occurs. This inhibition occurs in a characteristic fashion with the net results being a decrease in respiratory rate. First it was verified that a perfect correlation existed between this response in animals and the response in humans, i.e., complaints of eye, nose and throat irritation (*5, 6*).

Potency of These Chemicals

It was found that the decrease in respiratory rate was dependent on the exposure concentration of each chemical. By plotting the percentage decrease in respiratory rate versus the logarithm of the exposure concentration, a linear relationship was obtained (*4-6*). Such relationships are summarized in Figure 1 for 25 chemicals. From these relationships the exposure concentration necessary to evoke a 50% decrease in respiratory rate was obtained and termed RD_{50}. These values are given in Table 2. Thus the potency of these chemicals can be compared on the basis of their RD_{50} and

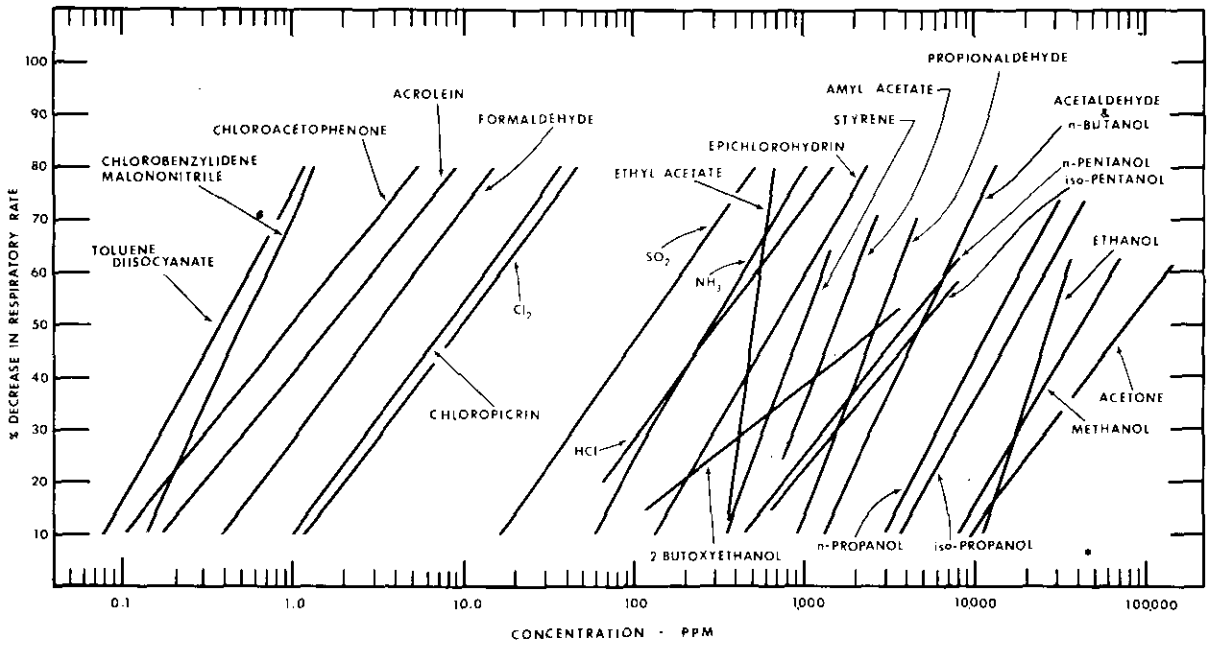

FIGURE 1. Concentration-response relationships obtained for 25 airborne chemicals. Data points are omitted for clarity but can be obtained from Kane (*6*, *8*) with the exception of styrene and amyl acetate. The concentration is given in ppm for all chemicals although in some cases an aerosol form was used.

Table 2. RD_{50} values, 95% confidence intervals, and regression equation for 25 airborne sensory irritants.

Compound	RD_{50}, ppm	95% confidence intervals, ppm	Regression equation $y = a + b \log x$
Acetaldehyde	4,946	4,579 – 5,381	–198.34 + 67.22
Acetone	77,517	59,004 – 115,366	–163.46 + 43.66
Acrolein	1.68	1.26 – 2.24	41.16 + 39.44
Ammonia	303	159 – 664	–94.27 + 58.13
Amyl acetate	1,531	1,295 – 1,902	–214.56 + 83.06
n-Butanol	4,784	3,797 – 7,727	–204.81 + 69.25
2-Butoxyethanol	2,824	1,695 – 7,278	–38.88 + 25.76
Chlorine	9.34	6.64 – 14.1	8.00 + 43.30
Chloroacetophenone	0.96	0.766 – 1.26	50.64 + 40.36
Chlorobenzylidene malononitrile	0.52	0.429 – 0.677	70.70 + 73.81
Chloropicrin	7.98	6.22 – 10.6	9.54 + 44.87
Epichlorohydrin	687	633 – 748	–108.58 + 55.90
Ethanol	27,314	24,154 – 32,605	–401.71 + 101.82
Ethyl acetate	614	562 – 684	–268.99 + 114.41
Formaldehyde	3.13	2.54 – 3.97	28.21 + 43.91
Hydrogen chloride	309	281 – 410	–59.40 + 43.95
Isopentanol	41,514	32,939 – 58,633	–207.16 + 55.68
Isopropanol	4,452	2,885 – 12,459	–109.95 + 43.84
Methanol	4,039	3,113 – 6,033	–99.20 + 41.37
n-Pentanol	17,693	15,509 – 20,511	–201.30 + 59.16
n-Propanol	12,704	11,558 – 14,152	–219.67 + 65.71
Propionaldehyde	2,751	2,294 – 4,009	–228.16 + 80.87
Styrene	980	826 – 1,297	–219.88 + 90.22
Sulfur dioxide	117	107 – 128	–47.06 + 46.95
Toluene diisocyanate	0.39	0.345 – 0.446	73.82 + 58.01

predictions made from this value. This is true if the curves are parallel or various corrections can be used. Inspection of Figure 1 (and the regression equations yielding the value for the slopes) reveals that the main exceptions are ethyl acetate, 2-butoxyethanol and ethanol. However, no correction will be made here; we will simply accept the RD_{50} values and proceed from there.

Prediction of Level of Response in Humans

Following the approach in Table 1, levels and types of effects expected to occur in humans at various RD_{50} ratios are presented in Table 3. To verify how close these predictions were, an extensive literature search was conducted for the first eleven chemicals tested (*6*, *7*); in general, a good correlation was found. Also for 19 of the 23 chemicals listed in Table 1 for which TLVs have been established, correct predictions were made for the range 0.01 to 0.1 RD_{50} as being where the value for the TLV should be (*6*, *8*). For one of the chemicals tested, sulfur dioxide, the highest level permitted for an Air Quality Standard was 0.1 ppm according to the model. The current Air Quality Standard is 0.03 ppm on the basis of annual average and 0.14 ppm for a 24-hr period. Perhaps a better way of looking at the data is to take a particular fraction of RD_{50}, a single value, instead of predicting an acceptable range. To do this, a convenient value would be 0.03 RD_{50}, the midpoint on a logarithmic scale, between the range 0.01 and 0.1 RD_{50}. These values are also listed. Taking the logarithm of both values and performing a regression analysis yields the results in Figure 2. If we believe the model to be correct, the TLV for formaldehyde and ethyl acetate should be reduced. However, what is rather remarkable is that the model seems to be working over five orders of magnitude of potency for the wide variety of chemicals tested.

Table 3. Predictions of level and type of responses in humans at various multiples of RD_{50} value found in mice.

Multiples of RD_{50}	Response
10	Severe injury, possibly lethal
1	Intolerable to humans
0.1	Some sensory irritation
0.01	No sensory irritation
0.001	No effect of any kind on respiratory system

Table 4. RD_{50}, Current TLV-TWA and Predicted Acceptable TLV-TWA on the Basis of 0.03 RD_{50}

Chemical	RD_{50}, ppm	1978 TLV-TWA, ppm	0.03 RD_{50}, ppm	log 1978 TLV-TWA, ppm	log 0.03 RD_{50}, ppm
Toluene diisocyanate	0.2	0.02	0.006	–1.70	–2.22
Chlorobenzylidene malononitrile	0.52	0.05	0.016	–1.30	–1.80
Chloroacetophenone	0.96	0.05	0.03	–1.30	–1.52
Acrolein	1.68	0.1	0.05	–1.00	–1.30
Formaldehyde	3.13	2.0	0.10	0.30	–1.0
Chloropicrin	7.98	0.1	0.25	–1.0	–0.602
Chlorine	9.34	1	0.30	0	–0.523
Sulfur dioxide	117	5	3.8	0.69	0.58
Ammonia	303	25	30	1.4	1.48
Hydrogen chloride	309	5	9.6	0.69	
Ethyl acetate	614	400	19.0	2.6	1.28
Epichlorohydrin	687	5	22	0.69	1.34
Styrene	980	100	31	2.0	1.49
Amyl acetate	1,531	100	48	2.0	1.68
Propionaldehyde	2,751	–	88	–	–
2-Butoxyethanol	2,825	50	89	1.7	1.95
n-Pentanol	4,039	–	129	–	–
Isopentanol	4,452	100	142	2.0	2.15
n-Butanol	4,784	50	152	1.7	2.18
Acetaldehyde	4,946	100	151	2.0	2.2
n-Propanol	12,704	200	402	2.3	2.6
Isopropanol	17,693	400	559	2.6	2.75
Ethanol	27,314	1000	864	3.0	2.94
Methanol	41,514	200	1,312	2.3	3.12
Acetone	77,516	1000	2,451	3.0	3.39

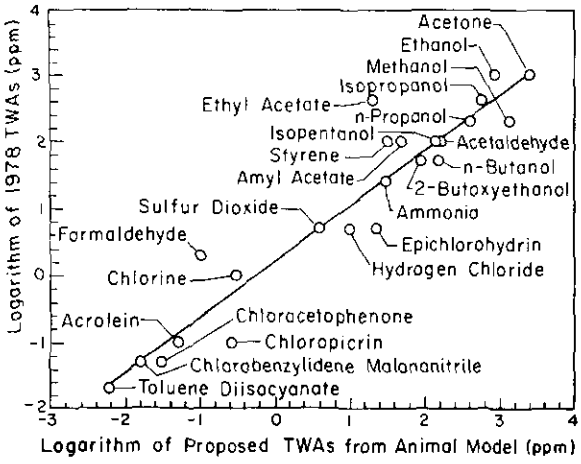

FIGURE 2. Regression analysis for 23 airborne chemicals obtained by plotting 0.03 RD_{50} as the proposed TLV-TWA versus the TLV-TWA for each chemical from the data given in Table 4. Regression equation, $y(x) = 0.24 + 83x$, $r = 0.95$.

Conclusions

In the case of these airborne contaminants it appears that the "appropriate" model has been selected and that "reasonable" predictions can be made for effects in humans over a wide range of concentrations and duration of exposure. Thus, this short-term test can be used to rapidly evaluate new as well as old chemicals never tested before to obtain their potency and make comparisons with the chemicals already tested. From these results, we can get a reasonable estimate of the level of control likely to be needed for industrial production and the cost for such controls. It is, at least, a starting point. The results can also be used for planning repeated exposures (*9*) to verify the appropriateness of the predictions and make necessary corrections prior to long term chronic studies.

The main problem in toxicology does not seem to me to be related to dose-response analysis but rather to be related to the appropriateness of the models we use. Perhaps the new generation of toxicologists will concern themselves more with resolving this issue.

Written under support from Grant OH-00367 from the National Institute of Occupational Safety and Health.

REFERENCES

1. Hunter, W. G., and Crowley, J. J. Hazardous substances, the environment and public health: a statistical overview. Environ. Health Perspect. 32: 241-254 (1979).
2. Gehring, P. J., Watanabe, P. G., and Park, C. N. Risk of angiosarcoma in workers exposed to vinyl chloride as predicted from studies in rats. Toxicol. Appl. Pharmacol. 49: 15-21 (1979).
3. Frazer, A. Pharmacological aspects of chemicals in food. Endeavour 12: 43-47 (1953).
4. Alarie, Y. Irritating properties of airborne materials to the upper respiratory tract. Arch. Environ. Health 13: 433-449 (1966).
5. Alarie, Y. Sensory irritation by airborne chemicals. CRC Crit. Rev. Toxicol. 2: 299-363 (1973).
6. Kane, L. E., Barrow, C. S., and Alarie, Y. A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Amer. Ind. Hyg. Assoc. J. 40: 207-229 (1979).
7. Alarie, Y., Kane, L., and Barrow, C. S. Sensory irritation: a basis to establish acceptable exposure to airborne chemical irritants. In: Principles and Practice of Industrial Toxicology, A. Reeves and H. N. MacFarland, Eds., Wiley, New York, in press.
8. Kane, L. E., Dombroske, R., and Alarie, Y. Evaluation of sensory irritation from some common industrial solvents. Amer. Ind. Hyg. Assoc. J. 41: 451-455 (1980).
9. Sangha, G. K., and Alarie, Y. Sensory irritation by toluene diisocyanate in single and repeated exposures. Toxicol. Appl. Pharmacol. 50: 533-547 (1979).