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BIOASSAY FOR EVALUATING THE POTENCY OF AIRBORNE SENSORY IRRITANTS AND PREDICTING ACCEPTABLE LEVELS OF EXPOSURE IN MAN

PLAINTIFF'S EXHIBIT

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Summary—An animal bioassay has been used to evaluate the potency of a wide variety of airborne sensory irritants. Concentration-response relationships were obtained for each chemical tested and the potencies of these sensory irritants were compared. An attempt was made to predict from the data obtained in the animal bioassay what the response would be in humans. A good correlation was found between the Threshold Limit Values for these chemicals and the level that, on the basis of the potency of each chemical, would be predicted to be acceptable in humans.

Introduction

In 1966, Alarie proposed that the trigeminal nerve endings in the nasal mucosa in mice could be used to detect whether or not airborne chemicals would have sensory irritating properties in humans. A sensory irritant was defined as a substance that would evoke a painful burning sensation of the eye, nose or throat in man (Alarie, 1966 & 1973). However, instead of measuring neural activity from these nerve endings directly during exposure to airborne chemicals (Kulle & Cooper, 1975; Ulrich, Haddock & Alarie, 1972) the response measured was a decrease in respiratory rate. A decrease in respiratory rate due to stimulation of the trigeminal nerve endings by airborne irritants was first described by Kratschmer in 1870.

First, it was shown for a series of chemicals (Alarie, 1966 & 1973; Kane, Barrow & Alarie, 1979) that a perfect correlation existed between the characteristic decrease in respiratory rate in mice and subjective reports of sensory irritation of the eyes, nose and throat in humans. Secondly, it was shown that concentration-response relationships were easily obtained by plotting the percentage decrease in respiratory rate *vs* the logarithm of the exposure concentration for each airborne irritant (Alarie, 1966 & 1981; Kane *et al.* 1979; Kane, Dombroske & Alarie, 1980). Finally a major step in the development of this model was to select for investigation in mice a series of eleven airborne industrial contaminants, which were well recognized as sensory irritants in humans and for which the Threshold Limit Value (TLV) had been established *almost entirely* to prevent complaints of sensory irritation in industry (Kane *et al.* 1979). It was expected that some relationship could be found between the TLVs of these chemicals and their RD_{50} values (the concentrations necessary to evoke a 50% decrease in respiratory rate).

In two recent reports (Kane *et al.* 1979 & 1980), such relationships were presented and evaluated, and in a preliminary report (Alarie, 1981) data have been presented showing that a good correlation could be obtained between the TLV values and 0·03 RD_{50} values for each chemical in a series ranging from very potent to very weak sensory irritants. In this report, the results obtained for 26 chemicals are presented and the value of this animal bioassay is discussed.

Method

The method for measuring respiratory rate in mice during exposure to airborne chemicals has been described in detail (Alarie, 1966; Barrow, Alarie, Warrick & Stock, 1977). Briefly, the tidal volume of each mouse is recorded by body plethysmography and displayed on an oscillograph, so that the characteristic pause during expiration can be observed as an indication that the net decrease in respiratory rate is due to stimulation of the nasal trigeminal nerve endings. The average respiratory rate of four mice is displayed on a second oscillograph which permits continuous monitoring prior to, during and following exposure to airborne chemicals. In order to obtain concentration-response relationships, five to eight groups of animals are used for exposure to various concentrations of each airborne chemical. From these relationships, the RD_{50} is obtained and a comparison of potency is made on this basis.

Prediction of acceptable exposure concentration in industry

The chemicals previously tested are presented in Table 1 in order of descending potency. As a prediction for an industrial TLV, it was originally proposed that the TLV be set between 0·01 and 0·1 RD_{50} (Barrow *et al.* 1977). This suggestion was modified on the basis of the finding that, with repeated exposures at concentrations just above 0·1 RD_{50}, hypersusceptibility or cumulative effects could be detected for some chemicals such as acrolein, formaldehyde and toluene diisocyanate (Kane & Alarie, 1977; Sangha & Alarie, 1979). Therefore 0·1 RD_{50} was proposed as a ceiling level (Sangha & Alarie, 1979).

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Table 1. *The RD_{50} values, 1980 TLV-TWA values* and TLV-TWA values predicted on the basis of 0·03 RD_{50} for 26 industrial chemicals*

Chemicals tested	RD_{50} (ppm)	1980 TLV-TWA (ppm)	0·03 RD_{50} (ppm)	log 1980 TLV-TWA (ppm)	log 0·03 RD_{50} (ppm)	Reference providing RD_{50} value
2,4-Toluene diisocyanate	0·20	0·005	0·006	−2·30	−2·22	Sangha & Alarie, 1979
Chlorobenzylidene malononitrile	0·52	0·05	0·016	−1·30	−1·80	Kane *et al.* 1979
Chloroacetophenone	0·96	0·05	0·029	−1·30	−1·54	Kane *et al.* 1979
Acrolein	1·68	0·1	0·05	−1·00	−1·30	Kane & Alarie, 1977
Formaldehyde	3·13	2	0·10	0·30	−1·00	Kane & Alarie, 1977
Benzoquinone	5·0	0·1	0·15	−1·00	−0·82	Y. Alarie, unpublished data 1979
Chloropicrin	7·98	0·1	0·24	−1·00	−0·62	Kane *et al.* 1979
Chlorine	9·34	1·0	0·28	0	−0·55	Barrow *et al.* 1977
Sulphur dioxide	117	2·0	3·5	0·30	0·54	Alarie, Wakisaka & Oka, 1973
Ammonia	303	25	9·1	1·40	0·96	Barrow, Alarie & Stock, 1978
Hydrogen chloride	309	5	9·3	0·70	0·97	Barrow *et al.* 1977
Dimethylamine	511	10	15	1·00	1·19	Steinhagen, Swenberg & Barrow, 1981
Ethyl acetate	614	400	18	2·60	1·26	Kane *et al.* 1980
Epichlorohydrin	687	2	20	0·30	1·31	Kane *et al.* 1979
Styrene	980	50	29	1·70	1·47	Y. Alarie, unpublished data 1979
Amyl acetate	1531	100	46	2·00	1·66	Kane *et al.* 1980
2-Butoxyethanol	2825	25	85	1·40	1·92	Kane *et al.* 1980
Isoamyl alcohol	4452	100	134	2·00	2·13	Kane *et al.* 1980
n-Butyl alcohol	4784	50	143	1·70	2·15	Kane *et al.* 1980
Acetaldehyde	4946	100	148	2·00	2·17	Kane *et al.* 1980
Methyl ethyl ketone	9000	200	270	2·30	2·43	Stone, Lawhorn, McKinney & McCracken, 1981
n-Propyl alcohol	12,704	200	381	2·30	2·58	Kane *et al.* 1980
Isopropyl alcohol	17,693	400	531	2·60	2·73	Kane *et al.* 1980
Ethanol	27,314	1000	819	3·00	2·91	Kane *et al.* 1980
Methanol	41,514	200	1245	2·30	3·10	Kane *et al.* 1980
Acetone	77,516	750	2325	2·88	3·37	Kane *et al.* 1980

TLV-TWA = Threshold Limit Value, time-weighted average

*When notice of intended change has been given (ACGIH, 1980) the intended future value is used. All values in the table are given in ppm for purposes of comparison, although for some chemicals the aerosol form instead of the gas phase was tested and the TLV-TWA would be given in mg/m³.

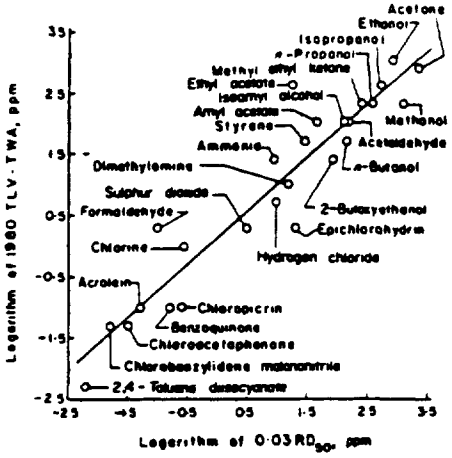

Fig. 1. Linear least squares regression analysis for 26 chemicals, plotting log of 0·03 RD_{50} as the proposed TLV-TWA *vs.* log of TLV-TWA for each chemical. Data are taken from Table 1. Regression equation: $Y = 0{\cdot}126 + 0{\cdot}865\,X$. Standard deviation of Y about the regression line = 0·507, r^2 = 89·4%, r^2 adjusted for 24 degrees of freedom = 88·9%.

Since TLVs are established as a time-weighted average (TWA) concentration for a normal 8-hr workday, a suggestion was made that 0·03 RD_{50} be used as a TLV-TWA. This value, which on a logarithmic scale is the mid-point in the proposed range of 0·01–0·1 RD_{50}, was selected for practical reasons to prevent, as much as possible, excursions above 0·1 RD_{50} in industrial situations. If 0·03 RD_{50} is selected for use in predicting an acceptable TLV-TWA, a good relationship should exist between this value and the TLV-TWA for these chemicals. This should be especially apparent in instances in which the primary basis for establishing the TLV-TWA was to prevent sensory irritation. Such a relationship is presented in Fig. 1 using the data in Table 1 for each chemical tested.

The relationship was found to be good for this very wide variety of chemicals, which cover a potency range of five orders of magnitude. There are two compounds for which the animal model predicts a much lower TLV-TWA than the current one; these are formaldehyde and ethyl acetate. However, the documentation (American Conference of Governmental Industrial Hygienists—ACGIH, 1980) clearly recognizes that for these two chemicals, sensory irritation will occur *at* the TLV-TWA currently set. The relationship in Fig. 1 indicates one instance (epichlorohydrin) where the TLV-TWA is much lower than would be predicted by the model. The TLV-TWA was recently reduced for epichlorohydrin (ACGIH, 1980) on the basis of systemic toxicity.

Application of the model

This model can be useful in predicting a level of exposure likely to be acceptable in the industrial situation to prevent sensory irritation in humans. It would be foolish to think that a TLV can be established on the basis of this test alone. On the other hand, the results of this test indicate the maximum value likely to be acceptable for a TLV and can be used for engineering design and controls needed for the production of the chemical.

The results from this test can also be used for the design of longer-term inhalation studies. The 0·01 RD_{50} can be used as the low level and multiples of it for the higher levels. However, it is unlikely that levels approaching the RD_{50} will be of value for long-term inhalation studies. At this level, and above, the respiratory rate is depressed to a point where CO_2 retention is significant and important changes in acid-base balance occur. Since the RD_{50} concentration results in intolerable sensory irritation in humans (Kane *et al.* 1979), exposing animals to such levels in long-term chronic studies seems inappropriate. However, with several chemicals a fade in the sensory irritation response occurs in man as well as in mice. In these cases the decrease in respiratory rate occurring at the beginning of exposure diminishes with time. This occurrence was observed with sulphur dioxide, ammonia, formaldehyde and many of the solvents listed in Table 1. In such instances, it would be possible to use RD_{50} levels for long-term inhalation studies.

With other irritants, such as 2,4-toluene diisocyanate, benzoquinone and a series of isocyanates, the depression of respiratory rate was long lasting following a 3-hr exposure. With benzoquinone the respiratory-rate depression was long lasting following exposures as brief as 30 min. Repeated daily exposure to these chemicals at and above the 0·1 RD_{50} will result in cumulative effects, the net result being a depression in respiratory rate of 80% after 3–5 days of exposure (Sangha & Alarie, 1979; Sangha, Matijak & Alarie, 1981). This is an extremely stressful situation for the animals and would not be recommended for long-term studies. So far the model has been used primarily for single exposures. The few experiments conducted with repeated exposures, such as with the isocyanates noted above, indicate that much more can be learned about their toxicity with such protocols, and possible corrections could be made to allow the use of 0·03 RD_{50} to predict safe levels of exposure.

The model has also been used for evaluating the potency of a series of surfactants used in hair shampoos (Ciuchta & Dodd, 1978). The objective was to determine which of the detergents was the most or least potent sensory irritant by exposing mice to various airborne concentrations of each. Excellent concentration-response relationships were obtained and valid comparisons were made with eye-irritancy tests. Although the animal model measures sensory irritation caused by stimulation of nerve endings in the nasal mucosa, it should be remembered that the cornea is endowed with similar trigeminal nerve endings, although their anatomical location is slightly different (Kane & Alarie, 1977). A chemical that stimulates the nasal trigeminal nerve endings will also stimulate comparable nerve endings located in the cornea (Alarie, 1973). Thus the model can be used to evaluate irritation by cosmetic ingredients providing the ingredients can be converted to aerosols for the evaluation of their potential as sensory irritants. In fact, this animal model could be 'calibrated' in a way similar to that used to 'calibrate' the model for TLVs, by testing a series of ingredients used in cosmetics for which there are data on consumer complaints of eye irritation. However, the model would not replace the

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eye-irritancy tests used to study the corrosive action of chemicals applied to the eye.

Concluding remarks

The trigeminal nerve endings are the 'common chemical sense' receptors (Alarie, 1973). They are stimulated by potentially noxious chemicals to warn man about the presence of such chemicals in his environment. In effect, they seem to 'measure' the reactivity of chemicals with nucleophiles (Alarie, 1973); the higher their reactivity the more potent the chemicals are as sensory irritants. It is therefore not so strange that if the concentration of a chemical is maintained at a level below which sensory irritation occurs, the likelihood that toxic effects will occur will be small. The fact that TLV levels established to prevent complaints of sensory irritation *seem* to be adequate to prevent the occurrence of other toxic effects from these chemicals gives support to this general idea. The main disadvantage of the model would be for chemicals that are of low reactivity but are metabolically activated. For these chemicals, as well as for ozone, phosgene and nitrogen dioxide, which are pulmonary irritants rather than sensory irritants, the model may predict a TLV-TWA that would be too high. This is under investigation. Another important point to consider is that the relationship presented in Fig. 1 was based on a single determination of the RD_{50} for each chemical. It would be more appropriate to have several determinations from different laboratories using chemicals of known purity.

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