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Use of Bioassays in Assessing Health Hazards from Complex Mixtures: A RASH Analysis¹

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October 14, 1993

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Abstract: The Finney harmonic mean model for joint toxicity of ingredients in mixtures can be used to estimate the toxicity of the neat compound if one component can be substituted in potency-adjusted-doses for each of the other components. Chemical analysis data and relative potency values (computed according to the RAPid Screening of Hazard (RASH) method) were used to compare the toxicities as predicted from ingredients of cigarette smoke, PAHs in diesel exhaust, asphalt, coal tar, pitch, and creosote with the measured toxicities of the neat mixtures. Accuracy for cigarette smoke condensate, coal tar, pitch, and creosote were within a factor of three; asphalt within a factor of 18; but the PAC content of diesel particulate was inadequate to accurately describe the toxicity of diesel emissions.

Use of Bioassays in Assessing Health Hazards from Complex Mixtures: A RASH Analysis¹

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Background: Frequently B(a)P has been used as a reference compound for chemical exposures and this convention has been continued in the RAPid Screening of Hazard (RASH) analogy (Jones et al. 1985). In addition, RASH has used other compounds as secondary standards in order to reduce uncertainty due to data gaps and to uncharacteristic response from a particular bioassay and chemical combination (Owen and Jones 1990; Jones et al. 1991).

The increased risk--perhaps cancer--as a result of a reference dose of B(a)P is

$$R_{B(a)P} = S_{B(a)P} \times D_{B(a)P}, \quad (1)$$

where $S_{B(a)P}$ is a risk coefficient for B(a)P and $D_{B(a)P}$ is the dose. For an unassessed hazard "i," the same functional dose-response may be postulated. The objective is to compute an effective dose of the interviewing hazard in terms of the reference hazard. The form becomes

$$R_i = S_{B(a)P} \times RP_{i/B(a)P} \times D_i, \quad (2)$$

where $RP_{i/B(a)P}$ is the strength of hazard i relative to B(a)P, and D_i is the dose of the interviewing hazard. Different doses of different compounds are associated with equal levels of risk, $R_{B(a)P} = R_i$ so the potency of i relative to B(a)P is

$$RP_{i/B(a)P} = D_{B(a)P}/D_i, \quad (3)$$

Relative potency can be used to convert a concentration of an interviewing hazard, C_i , to a hypothetical equivalent-toxic-concentration of the reference compound, C_R , according to

$$C_R = C_i \times RP_{i/R}. \quad (4)$$

If C_R is set as the "permissible" concentration of the reference agent, then

$$C_i = C_R/RP_{i/R} \quad (5)$$

would be an estimate of the permissible concentration for any chemical hazard i if the implicit level of hazard for exposure to hazard R is also acceptable for exposure for exposure to hazard i.

Action of Complex Mixtures: Finney (1971) reviewed three types of joint toxic actions: (1) **independent** if poisons or drugs have different modes of action; (2) **similar** if one poison or drug can be substituted in a constant proportion for the other; and (3) **synergistic** if the effectiveness of the mixture cannot be assessed from that of the ingredients. Ingredients may alter (1) absorption into the body, (2) vascular distribution, (3) detoxification, (4) elimination, or (5) organ sensitivity.

Possible synergisms have stimulated interactive terms in dose-response models since the 1930s, but we have not progressed beyond Finney's simple observation that for synergism "the effectiveness of the mixture cannot be assessed from that of the individual ingredients . . ."

For independent and similar modes of joint action, the toxicity of the mixture is predictable to a useful degree from concentrations and toxicities of the ingredients. Finney noted that the potency of a mixture whose ingredients act similarly is generally greater than that of a mixture in the same proportion, having constituents that are of the same individual potencies but act independently. The effect could derive from the "hockey-stick" shaped dose responses for ingredients. For a common response mechanism, an incremental dose should be added to the primary dose so that the change is incremental along a reference curve in contrast to the result obtained by summing initial increments from different curves.

Dose-Additive Effects: If agent 1 contributes dose d_1 and agent 2 contributes dose d_2 , the combination becomes $R_1\{d_1\} + [R_1\{d_1 + (RP_{2/1})d_2\} - R_1\{d_1\}]$ or $R_1\{d_1 + (RP_{2/1})d_2\}$. (6)

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Similar Joint Action of Mixtures: For a single compound, the effect E (or response from Eqn. 1) is equal to the risk coefficient (S) times the dose (D) times the relative potency (RP). If the dose is comprised of two fractions f_1 and f_2 of compounds 1 and 2,

$$\text{dose} = (f_1 + f_2)D \quad (7)$$

$$\text{and} \quad E_1 = S(f_1 D)RP_1, \text{ and } E_2 = S(f_2 D)RP_2. \quad (8)$$

The composite effect is then $E = E_1 + E_2$, so that

$$S(RP_{\text{mix}})D = E_1 + E_2. \quad (9)$$

$$\text{Therefore,} \quad S(RP_{\text{mix}})D = S(f_1 D)RP_1 + S(f_2 D)RP_2, \quad (10)$$

$$\text{or,} \quad RP_{\text{mix}} = f_1(RP_1) + f_2(RP_2). \quad (11)$$

For n components of an exposure, the relative potency becomes,

$$RP_{\text{mix}} = \sum f_j(RP_j) \quad (12)$$

where index j ranges from 1 to n.

Owen and Jones (1990) used 37 compounds of tobacco smoke (DHEW 1979) to predict a harmonic-mean based potency of 0.0128 relative to B(a)P. This estimate was based on gas and particulate fractions. For cigarette smoke tested as a neat compound, a relative potency of 0.0049 was computed.

Obviously, many such examples are needed, but it is rare to find adequate characterization data on mixtures. The IARC (1986) also compiled concentrations of biologically active agents in nonfilter cigarette mainstream smoke but listed 54 compounds. IARC's median concentrations were taken; updated RASH RPs based on newer bioassay data were used; and Finney's harmonic mean was recomputed.

Masses in the IARC smoke sum to 49.4 mg/cigarette in contrast to our previous value of 98 mg/cigarette, but that earlier estimate included 50.6 mg/cigarette of carbon dioxide. If the mass of condensate per cigarette is taken as 49.4 mg/cigarette plus the 50.6 mg/cigarette of CO_2 , then the harmonic mean estimate is 1.25 mg equivalent of B(a)P per 100 mg of mixture yielding a potency of 0.0125 as compared with 0.0049 for the neat compound. If the toxicological potency of CO_2 is added to the IARC concentrations, the newer estimate of the relative potency of cigarette smoke becomes about 0.0135. These values differ insignificantly from our previous estimate of 0.0128.

Diesel Exhaust Particulate: Transportation diesels commonly produce 0.5 to 1 g of particulates per mile (Hites et al. 1980; Westerholm et al. 1991). About 10 to 40% by weight is extractable with organic solvents. This fraction is mutagenic with or without metabolic activation (Hites et al. 1980). If the emission of particulate-associated PACs is taken from Westerholm et al. (1991) for a heavy-duty diesel exhaust, and the corresponding particulate emission of 0.62 g/km is used, then the harmonic mean potency is 0.000092. In an earlier study, Stenberg, et al. (1983) assayed PAH compounds on particles from a 2.3 L diesel. For the Stenberg light diesel, it was considered that 0.5 g of particulates are emitted per km, and the harmonic mean potency was estimated to be 0.00015. Thus, it appears that the PACs/PAHs comprise about 0.037 to 0.070% by weight and about 0.92 to 1.5% by reactivity. The PAC fraction of exhaust particulates does not seem to characterize the toxicity of the neat mixture accurately, but that has also been observed by Lewtas (1989) who used bioassay-directed fractionation to measure mutagenic activity.

Asphalt, Pitch, Coal Tar, and Creosote: Dailey (1977) compiled information and data on PAHs in asphalt, coal tar pitch, coal tar, and creosote. Median concentrations for ingredients were taken from the ranges provided, and RASH RPs were used to compute the predicted potency from ingredients in each mixture. Relative potencies were: asphalt (0.000021), coal tar pitch (0.060), coal tar (0.024), and creosote (0.027). In contrast, the relative potency values based on the complete mixtures were: asphalt (0.00038), coal tar pitch (0.044), coal tar (0.042), and creosote (0.016).

Conclusions: From calculations in Table 1, the harmonic mean model seems accurate for complex mixtures deriving from cigarette smoke, coal tar pitch, coal tar, creosote, and for the immense numbers of simple combinations tested by Pozzani et al. (1959), and Smyth et al. (1969, 1970). If characterization data were available for more complex mixtures, deviations might be significantly greater than found in these examples. However, even if the variations were 100-fold larger, that difference should not

discourage more extensive evaluation of the harmonic mean formula for screening and comparing hazards from complex mixtures.

For carcinogens, the relative potency for each of the 58 EPA-CAG carcinogens was evaluated by the RASH method. Those values define a log-probability distribution for "carcinogens." Upon that background, epidemiologically defined carcinogens benzene, arsenic, coke oven emissions, benzidine, and chromium VI are shown as reference points. In addition, ethyl alcohol and vinyl chloride (animal-based) have been added to help calibrate the low end of the distribution--placement of all compounds in Fig. 1 is by the RASH evaluation.

Similarly, the distribution of RASH-RPs for 278 compounds (including carcinogens and noncarcinogens) were indexed by chromium, ozone, mercury, pentachlorophenol, lead, cresol, toluene, and kerosene. The RPs of the complex mixtures are compared on this background in Fig. 2.

Table 2. Summary of Harmonic Mean Predictions from Ingredients in Mixtures Compared with the Bioassay Toxicities of the Neat Mixtures.

Complex Mixture	Quantity Considered	Predicted RP	Measured RP	Reference
Cigarette Smoke ^d	37 of > 3800 compounds	0.0128	0.0049	DHEW 1979
Cigarette Smoke	53 of > 3800 compounds	0.0135	0.0049	IARC 1986
Asphalt ^e	PAH/Total	0.000021	0.00038	Dailey 1977
Coal Tar Pitch	PAH/Total	0.060	0.044	Dailey 1977
Coal Tar	PAH/Total	0.024	0.042	Dailey 1977
Creosote	PAH/Total	0.027	0.016	Dailey 1977
Heavy Diesel ^f	PAC/Total Particulate	(0.000092) ^g	0.010	Westerholm 1991
Light Diesel	PAC/Total Particulate	(0.00015) ^g	0.010	Sternberg 1983

^d Owen and Jones 1990 ^e Polyaromatic Hydrocarbons ^f Polyaromatic Compounds

^g Lewtas (1989) found that "very little" of the mutagenic activity was in the aromatic fraction.

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Fig 1. The cumulative distribution of RASH potencies is for the 58 carcinogens considered by the EPA-CAG. RASH-RPs for epidemiologically-based carcinogens, ethyl alcohol, and vinyl chloride are shown as reference points at the top of the figure. Potencies for mixtures (at the bottom) and can be compared with the distribution or the reference compounds at the top.

Fig 2. The cumulative distribution is for RASH-RPs for 278 common compounds comprised of carcinogens and noncarcinogens. Selected reference compounds are compared at the top. The mixtures are shown at the bottom and can be compared either with the distribution or the reference compounds at the top.

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