CHAPTER 9

MATHEMATICAL MODELS FOR CHEMICAL INTERACTIONS

Mathematical models can be used to describe a variety of biological phenomena that cannot be characterized adequately in other ways. These models describe relationships among variables and can thereby provide better insight into basic biological phenomena and their implications.

In the biological sciences, interests often extend into dose ranges or other areas where experimental measurement is not practical. In these situations we can use models that fit the data in the observable range to extrapolate into the nonobservable range. Although more than one model may fit the observable data, the extrapolations that they suggest may differ substantially. Because of these possible discrepancies, it is highly desirable that a model have a biological basis. The models can, of course, be refined or extended as new information becomes available.

EXPLANATION OF THE ONE-HIT MODEL

The "one-hit" model has been used to describe the dose-response relationships of agents that produce cancer in humans. For cancer, a "hit" can be conceptualized as a permanent "change in cellular genetics resulting from interaction of one molecule of carcinogen with a critical receptor in one cell" (Maugh, 1978).

In general, if, at dose D, λD is the expected number of "hits", then the probability of exactly x hits is described by the Poisson Probability Law:

$$P[X = x] = \frac{e^{-\lambda D}(\lambda D)^x}{x!}$$

where e is exposure and λ is an unknown constant. The one-hit model assumes that only one hit is required to produce the disease response. Therefore, the probability (P) of response at dose D is:

$$P(D) = P[x \geq 1] = 1 - e^{-\lambda D}$$

P(D) can also be described as the proportion of individuals who would respond to dose D. Note that the one-hit model is essentially equivalent to the assumption of a linear dose-response relationship at low dosages, that is, $P(D) = \lambda D$ for low doses.

The one-hit model sometimes fits actual data reasonably well in the lower doses of the observable range. Various authors have argued that it is consistent with reasonable biological assumptions. However, others maintain that the one-hit model, or linear model at low doses, may not be adequate to explain all forms of carcinogenesis.

Several authors have proposed more complex models for these phenomena. In 1976, the Environmental Protection Agency (EPA) specified this model in its interim guidelines for risk assessment for suspected carcinogens.

INDEPENDENT ACTION MODEL vs. INTERACTION

The independent action model describes chemicals in a mixture whose joint effect is additive, i.e., the effect is neither more nor less than the sum of the independent effects of each chemical. This can be illustrated by the one-hit model. Assume that an individual is exposed to chemical A alone at dose D_1 and that this chemical follows the one-hit model in which $\lambda_1 D_1$ is the expected number of hits. Also assume that another individual is exposed to chemical B alone at dose D_2 and that this chemical follows the one-hit model in which $\lambda_2 D_2$ is the expected number of hits. If a person is exposed to both chemicals A and B at doses D_1 and D_2, respectively, and this mixture follows the one-hit model so that the expected number of hits is $\lambda_1 D_1 + \lambda_2 D_2$, then chemicals A and B are said to have independent action. That is, assuming a one-hit model for substance A and B and assuming independent action of A and B,

$$P(D_1,D_2) = 1 - e^{-\lambda_1 D_1 - \lambda_2 D_2} \quad \text{or}$$

$$P(D_1,D_2) = \lambda_1 D_1 + \lambda_2 D_2$$

for low doses.

The idea of independent action can also be generalized to other models. For example, if $P(D_1,D_2)$ is the probability of response for individuals with exposure to both chemicals A and B at doses D_1 and D_2, respectively, then an independent action model is characterized as:

$$P(D_1,D_2) = P(D_1) + P(D_2) - P(D_1)P(D_2) \quad (1)$$

In contrast to independent action, interaction, i.e., synergism or an antagonism, is said to occur when the combined effect of chemicals is greater or less than the sum of the independent action of each chemical. Three indices that measure the degree of interaction are related to equation (1) (Finney, 1971; Hogan et al., 1978; Walter, 1976);

$$I_H = R(D_1,D_2) - R(D_1) - R(D_2) + 2, \quad (2)$$

$$I_F = \frac{R(D_1,D_2) - 1}{R(D_1) + R(D_2) - 2}, \text{ and} \quad (3)$$

$$I_W = \frac{R(D_1, D_2)}{R(0_1,D_2)\ R(D_1 0)} \quad (4)$$

where I is the extent of interaction; H, F, and W refer to the Hogan, Finney, and Walter references, respectively; 0 refers to unexposed populations; and R(D) is the relative risk of individuals exposed to dose D compared to unexposed individuals, i.e.:

$$R(D) = \frac{P(D)}{P(0)}$$

Essentially, equations (2), (3), and (4) measure the degree to which equation (1), the criterion for independent action, is not true. Independence is indicated by values equal to 1, synergism by values greater than 1, and antagonism by values less than 1.

For low doses, the independent one-hit model results in values of approximately 1 for each index. Although these indices can be used to measure the degree of synergism or antagonism,

they may vary widely and yield conflicting results (Blot and Day, 1979; Hamilton and Hoel, 1978; Rothman, 1978a,b; Walter and Holford, 1978).

PARALLELISM

The property of parallelism can be defined formally as follows. If the probability of response to chemicals A and B at dose D are $P_A(D)$ and $P_B(D)$, respectively, then parallelism implies that:

$$P_A(D) = P_B(fD)$$

for all doses, where f is the relative potency of chemical A to chemical B.

In this model of parallelism, two chemicals acting alone respond as if one were a dilution of the other, dilution being defined as a mixture of two chemicals when one chemical is completely inert to the response of interest. This does not mean that one is in fact a dilution of the other but simply that the dose-response curves for these chemicals look like one could be a dilution of the other.

If two chemicals have the parallelism property and the chemicals act independently, then the transformed dose-response curve for the combined effect of these chemicals will be parallel to the curves for the individual chemicals. This is not necessarily the case for a model for independent action.

DOSEWISE ADDITIVITY

In a model of dosewise additivity, one chemical acts as if it were a dilution of the other but in a different way than discussed above (American Conference of Governmental Industrial Hygienists, 1977). For example, if 100 g of chemical A is equivalent in potency to 10 g of chemical B, this model indicates that a mixture of 50 g of A and 5 g of B would have the same potency as 100 g of A alone or 10 g of B alone. Furthermore, the mixtures--25 g and 7.5 g, 75 g and 2.5 g, 10 g and 9 g, and, in general, 100π and $10(1-\pi)$, where $0< \pi < 1$, would also have this same potency. Although chemical B is assumed to be 10 times as potent as A at a particular dosage, the dosewise additivity model does not guarantee that this particular relationship, the property of parallelism, will apply to other doses. On the other hand, the possession of this property by two chemicals does not in itself indicate what the efficacy of the mixtures might be.

Both parallelism and dosewise additivity have been described in terms of "apparent dilutions." However, if the dilutions were true, then both the parallelism and dosewise additivity properties should apply.

The independent action one-hit model has the property of dosewise additivity, but dependent versions of this model generally do not.

ACGIH THRESHOLD LIMIT CRITERION FOR EXPOSURE TO MORE THAN ONE CHEMICAL

The American Conference of Governmental Industrial Hygienists (ACGIH, 1977) has developed a threshold limit criterion for chemical mixtures or multiple exposures. When $C_i \equiv$ observed concentration for chemical i and $T_i \equiv$ the threshold limit value for chemical i for a mixture of n chemicals that affect the same body organs, if $\sum_{i=i}^{n} Ci/Ti > 1$, the threshold limit of the mixture is considered to be exceeded. Gart, whose paper is attached to this report as Appendix B, made three important points with respect to this criterion.

1. For low doses, this criterion can be justified if one assumes that the chemicals follow the independent one-hit model.
2. Under the conditions stated in 1, the ACGIH criterion can be extended to include mixtures of chemicals that affect different organ systems.
3. Under certain circumstances this criterion can be misleading when applied to low doses if the chemicals follow a one-hit model that does not have independent action.

Models other than the one-hit or linear model may be useful in actual practice. One may be engaged in linear extrapolations when the underlying reality is not linear. The conclusions drawn may either underestimate or overestimate the risk, depending on the circumstances.

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CHAPTER 10

LITERATURE SEARCH AND BACKGROUND ON INTERACTIONS

Preliminary attempts to locate articles dealing with toxicological interactions among 22 exemplary compounds listed by the Coast Guard (Table 11-1) involved searches in MEDLINE and TOXLINE under such headings as drug synergism, drug antagonism, and interactions. Although volumes have been written about potential drug-drug interactions (e.g., Newberne et al., 1978; Veldstra, 1956), there is a paucity of reports on interaction of common or widely used drugs and exposure to other chemicals of concern in marine occupations. Further search of the literature and communications with other scientists led to only a few additional reports.

Finally, all the references in another government data base were searched for the 11 compounds underlined in Table 11-1. All of these references have been incorporated into a single data base and constitute the presently available information on which the comments in this section are based. Certain well-known examples of interaction due to enzyme induction are not included because, for the most part, they did not relate to the compounds in Table 11-1 and have been adequately discussed in other sections of this report. Many reports that consider toxicological interactions are not retrievable by means of the usual key words intended to relate to interactions.

Published reports on the toxicity of various solvent-solvent or solvent-drug combinations can be divided into three groups. The first group contains those for which no synergism (only addition or summation) of effects was observed. The second group generally

Table 11-1

Candidate Materials of Interest to the U.S. Coast Guard as Possible Agents for Interaction Modeling

Compound	Molecular Weight	Boiling Point, °C	Melting Point, °C	Specific Gravity (°C)	Vapor Pressure 20 °C	Vapor Pressure 37 °C	Solubility: Water	Solubility: Ethanol	Solubility: Benzene	Threshold Limit Value, ppm	Short-Term Exposure Limit, ppm	Acute Inhalation Toxicity: Animal	Time of Exposure	LC50, ppm	References
Acetic Acid	60.05	117.5	16.7	1.050 (20)	0.95	0.60	Miscible	Miscible	Miscible	10	15	Guinea Pig	1 h	5,000	Ghiringhalli and Di Fabio, 1957
												Mouse	1 h	5,620	American Industrial Hygiene Association, 1972
Acetone	58.08	56.1	-94.7	0.791 (20)	4.0	7.5	Miscible	Miscible	Miscible	1,000	1,250	Mouse	3 h	130,000	Meklesova and Kudrina, 1969
Acrylonitrile	53.06	77.4	-83.6	0.807 (20)	1.8	3.5	Soluble	Soluble	Soluble	20(40)	(60)	Rat	4 h	500	Carpenter et al., 1949
Benzene	78.10	80.1	5.5	0.879 (20)	1.6	3.2	Slightly soluble	Miscible	-	10	(25)	Guinea Pig	4 h	576	Dudley and Neal, 1942
												Rat	4 h	16,000	Carpenter et al., 1949
n-Butyraldehyde	72.11	74.8	-96.4	0.803 (20)	2.8	6.0	Soluble	Miscible	Soluble	-	-	Rat	7 h	10,000	Gerarde, 1960
												Mouse	7 h	9,980	Svirbely et al., 1943
Carbon tetrachloride	153.83	76.5	-23.0	1.590 (20)	1.9	4.0	Slightly soluble	Soluble	Miscible	10	20	Rat	30 min	60,000	Fassett, 1963
												Mouse	6 h	7,329	Gradiski et al., 1978
Cresols (isomers)	108.13	177.0	-	1.030 (20)	0.13	0.35	Soluble	Miscible	Miscible	5	10	Rat	30 min	21,900	Spector, 1956
												Mouse	7 h	9,528	Svirbely et al., 1943
Cumene	120.19	152.4	-96.1	0.866 (15)	0.28	0.56	Slightly soluble	Soluble	Soluble	50	75	Rat	1 h	>161 [a]	Industrial Bio-Test Laboratories, Inc., 1969a
												Rat	1 h	>277 [b]	Industrial Bio-Test Laboratories, Inc., 1969a
Ethanol	46.07	78.3	-114.0	0.790 (20)	0.90	0.22	Miscible	-	Miscible	1,000	1,250	Rat	1 h	>4,510	Industrial Bio-Test Laboratories, Inc., 1969b
												Rat	4 h	8,000	Smyth et al., 1951
Ethanolamine (mono)	61.08	170.8	10.3	1.010 (25)	-	-	Miscible	Soluble	Soluble	3	6	Rat	4 h	10,000-12,000	Treon, 1963
Ethylene diamine	60.10	117.0	11.0	0.909 (20)	0.20	0.54	Miscible	Soluble	Slightly soluble	10	20	-	-	-	
Ethylene dibromide	187.86	131.0	9.8	2.180 (20)	0.20	0.50	Slightly soluble	Miscible	Soluble	20	30	Rat	8 h	4,000	Smyth et al., 1951
Ethylene dichloride	98.96	83.5	-35.7	1.253 (20)	1.4	2.2	Slightly soluble	Slightly soluble	Soluble	50	75	Rat	2 h	400 [c]	Rowe et al., 1952
												Rabbit	1 h	6,425 [c]	Irish et al., 1940
Ethylene glycol (vapor)	62.07	197.6	-13.0	1.115 (20)	0.014	0.050	Miscible	Soluble	Soluble	100	125	Guinea Pig	3 h	400 [c]	Rowe et al., 1952
												Mouse	6 h	262	Gradiski et al., 1978
Gasoline	-	60-199	-	0.71-0.74 (20)	-	-	Slightly soluble	Miscible	Miscible	-	-	-	-	-	
Methanol	32.04	64.5	-97.8	0.792 (20)	1.9	4.4	Miscible	Miscible	Miscible	200	250	Mouse	2 h	123mg/l [d]	Spector, 1956
												Cat	6 h	33,600	Treon, 1963
Isopropylamine	59.11	32.4	-95.2	0.889 (20)	-	-	Miscible	Miscible	Soluble	-	-	Mouse	3-4.5 h	242,000-363,000	Spector, 1956
Styrene	104.14	145.5	-30.6	0.909 (20)	-	-	Soluble	Soluble	Soluble	100	125	Rat	3-4.5 h	174,000	Spector, 1956
												Mouse	40 min	7,000	Shell Oil Co., 1961
Toluene	92.4	110.6	-95.0	0.867 (20)	0.65	1.0	Slightly soluble	Miscible	Miscible	100	150	Rat	4 h	8,000 [d]	Spector, 1956
												Mouse	2 h	5,000	Shugaev, 1969
Toluene diisocyanate	174.16	250.0	20-22	1.220 (25)	-	-	Slightly soluble	Miscible	Miscible	0.02	(0.02)	Rat	4 h	2,809	Shugaev, 1969
												Mouse	7 h	5,100	Svirbely et al., 1943
Trichloroethylene	131.39	87.0	-86.4	1.460 (20)	1.2	2.5	Slightly soluble	Miscible	Miscible	100	150	Mouse	2 h	7,980-9,310	Spector, 1956
Vinyl acetate	86.09	72.9	-92.8	0.934 (20)	1.9	3.0	Soluble	Soluble	Soluble	10	20	Rat	4 h	14	Duncan et al., 1962
												Mouse	4 h	10	Duncan et al., 1962
												Rabbit	4 h	8	Duncan et al., 1962
												Guinea Pig	4 h	13	Duncan et al., 1962
												Rat	1 h	26,000	Carpenter et al., 1949
												Rat	4 h	4,000	Svirbely et al., 1943

[a] m-, p-

[b] o-

[c] LCLo, lowest published lethal concentration.

[d] LC, lethal concentration.

includes reports of studies on combinations, some of which produce synergistic effects while others evidence no potentiation. A few reports specify a potentiating interaction between two agents. Although antagonistic interactions may reveal details of mechanisms, emphasis in this literature search was placed upon synergistic interactions in view of the concern of potentially increased (rather than decreased) hazard to marine personnel exposed to multiple vapors.

Drew and Fouts (1974) pretreated rats with phenobarbital and chlorpromazine and then administered benzene either by inhalation or by intraperitoneal injection (in mineral oil). They observed no potentiation of benzene toxicity. In a second study, Drew et al. (1978) examined the changes in serum enzyme levels in rats that had been given organic solvents either singly or in combination. They found that neither tetrachloroethylene in combination with dioxane, butyl ether, or acetonitrile nor a combination of trichloropropane and dichloropropane resulted in greater than an additive effect on the levels of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), or ornithine carbamyl transferase (OCT). In many instances the biological effects of solvent combinations were significantly less than additive. Adams et al. (1952) and McCollister et al. (1956) studied the toxicology of fumigant mixtures, principally carbon tetrachloride, ethylene dibromide, and ethylene dichloride, and observed either simple summation of the separate toxicities or only slight potentiation.

In two classic studies, Smyth et al. (1969, 1970) reported a 3-year study in which they explored the joint toxic action (based on

LD_{50}'s of 27 industrial chemicals. They first examined the joint action of 1:1 v/v mixtures of the solvents. Altogether, 350 combinations of solvents were studied. In the second paper they compared the results obtained using mixtures of equal toxicity versus equal volumes for 53 solvent pairs. It makes a considerable difference whether one administers a solvent on the basis of equal toxicity or equal volume. When data from equal volume experiments were expressed as expected/observed LD_{50} ratios, the nine pairs showing the greatest antagonistic effect had ratios of 0.23 to 0.40 (ratios of 1.0 indicate additive effects), whereas the nine pairs with the greatest apparent potentiating effects had ratios that varied from 2.7 to 5.09.

Shugaev (1969) studied combinations of such hydrocarbons as butane, butadiene, isoprene, and styrene. Three of the 12 combinations exhibited synergism (when the results are expressed as LC_{50}). In nine tests, only summation of the individual chemical effects was observed.

Deguchi (1972) examined the effects of single and combinations of chlorinated hydrocarbons on the levels of serum transaminases in rats. Greater than additive increases in SGOT and SGPT were observed following administration of carbon tetrachloride with trichloroethylene. Other combinations were less synergistic. Elevation in enzyme levels was dose related.

Ohtsuji and Ikeda (1971) found increased metabolism and toxicity in rats exposed to combinations of styrene and phenobarbital, and Saida et al. (1976) reported potentiation of peripheral nerve changes when a combination of methyl n-butyl ketone and methyl ethyl ketone was administered to rats and cats. The combination of carbon tetrachloride

and acetonitrile (Radimer et al., 1974) was more toxic to the skin than either component applied separately, and systemic effects including septic shock, upper gastrointestinal bleeding, hypotension, and anuria were also observed following exposure to the combination. Moon (1950) reported that another combination, carbon tetrachloride and ethyl alcohol, was more hepatotoxic and nephrotoxic in humans than was either organic solvent alone. This toxicological interaction has also been observed repeatedly in studies with animals (Cornish and Adefuin, 1966, 1967; Cornish et al., 1973; Klaassen and Plaa, 1967; Kotub and Plaa, 1962). Van Doorn et al. (1978) has recently shown that phorone (diisopropylideneacetone) exhibits synergistic hepatotoxic effects with bromobenzene or paracetamol in mice. Also, Larionov and Broitman (1975) found synergistic effects when 2,6-dimethylphenol and methanol were given simultaneously by inhalation.

Two studies have reported striking carcinogenic responses to chemical combinations that maritime personnel might encounter. When combinations of ethylene dibromide (EDB) and disulfiram (antabuse) were administered chronically to male and female Sprague-Dawley rats, Plotnick (1978) observed hemangiosarcomas of the liver, spleen, omentum, and kidney as well as adenocarcinoma of the mammary gland in females and a high incidence of testicular atrophy. Neither morbidity nor mortality was observed in groups receiving the same doses of EDB or disulfiram alone.

Radike et al. (1977) found that a low oral dose of alcohol administered chronically to rats receiving vinyl chloride by inhalation causes a more rapid and greater incidence of tumors.

Data from these studies indicate that many toxic effects produced by combinations of organic solvents or of solvents and drugs are additive. However, other combinations obviously produce synergistic effects. Consequently, it is important to develop methods or principles whereby the potentially hazardous effects of chemical combinations can be predicted for humans.

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CHAPTER 11

CONCLUSIONS AND RECOMMENDATIONS

In the course of its deliberations, the panel discussed at length the paucity of information regarding the health effects, exposure histories, and analytical data relevant to chemical exposures experienced by marine personnel. There was considerable discussion of action required to acquire such information. The recommendations listed below reflect these discussions and the panel's considerations of the need to develop basic principles for predicting the toxicological interactions that constitute the principle subject of this report. These recommendations for action are listed in order of highest priority, as judged by the panel. Until more specific information becomes available, it seems that the most productive course to follow to determine limits for multiple exposures is to assume additivity and follow the guidelines for mixtures as recommended by the American Conference of Governmental Industrial Hygienists (1977).

1. A better characterization of the causative agents and the potential added risk to health from multiple chemical exposures is essential. The panel recommends that the health evaluation studies of Coast Guard marine inspectors, which have already been initiated on the advice of the panel, be extended to include the following:

   - The specific chemicals and the frequency with which they are encountered during inspection should be identified. The concentrations, temperatures, and durations of exposure should be logged in a daily

record maintained by each inspector. Data on personal use of chemicals should also be recorded.

- Urinary analyses should be performed to establish the usefulness of excretion profiles of agents as indices of exposure.
- If indicated by the data resulting from the performance of the first two recommendations, biomedical tests, e.g., SMA 1260, myoneural conduction, pulmonary function, or central nervous system function tests, should be conducted on marine inspectors and their coworkers to detect evidence of injury to organ systems.

In conjunction with these studies, health records should be augmented by information concerning any unusual dietary habits and the use and abuse of drugs, alcohol, and tobacco.

2. Current analytical procedures used by marine chemists appear to be limited in scope and are generally semiquantitative. Therefore, the panel recommends that a program be devoted to the improvement of analytical capabilities of both marine inspectors and marine chemists. This program should include both educational programs and the provision of improved analytical instrumentation.

3. The panel recommends that a list of priority chemicals be identified. The selection of chemicals for this list should be based on the nature, extent, and frequency of occupational exposure of marine personnel. These compounds should then be subjected to:

- A comprehensive literature review to determine existing knowledge concerning biological effects and potential

toxicological interactions, including a search for data on possible exposure of humans to the same chemical in other industries and occupations.

- Experimental studies, where necessary, to extend the toxicological data base, including: research to obtain quantitative information regarding reaction kinetics at potential sites of interaction; determination of effects of exposures of laboratory animals to pairs of chemicals over a dosage range that includes the threshold limit values with assessments of effects selected on the basis of the known toxicological end points of the individual chemicals; and, where there is reason for special concern, more extensive toxicological tests, such as in-vitro assays for reactive intermediates and tests for myoneural conduction, altered behavior, two-stage carcinogenesis, mutagenesis, teratogenesis, effects on reproduction, and other tests as appropriate.

4. The Coast Guard should collaborate with organizations such as the National Library of Medicine, the Oak Ridge Toxicology Information Response Center, and other groups to improve collection, collation, and retrieval of toxicological data.

5. When unusually high risk of serious toxicological interaction is expected, the Coast Guard should develop criteria for classifying chemicals to protect against toxicological incompatibility.

6. The Coast Guard should expand its research studies and educational programs regarding chemicals and health to include a

broader segment of the marine industry by cooperating with unions, shipping companies, and other naval personnel.

7. When sufficient data have been collected, as recommended above, the Coast Guard should proceed to an assessment of potential interactions from combinations of specific materials to which marine personnel are frequently exposed.

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APPENDIX A

PANEL SITE VISIT -- HOUSTON SHIP CHANNEL

On January 11, 1979, the panel visited and inspected the M/T Stolt Sheaf which was loading cargo in the Houston Ship Channel in Texas. Built at a cost of approximately $40 million in 1972, this vessel is designed specifically for the transportation of bulk liquid cargoes. It is divided into five main cargo sections by transverse cofferdams, has double bottoms throughout, and a double hull for 65% of the cargo area. Transverse and longitudinal bulkheads are used to divide the cargo space into 40 separate tanks with liquid capacities ranging from 239 to 1,573 m^3. There are 9 stainless steel, 9 zinc-lined, and 22 phenolic-coated tanks, all of which could contain different chemical cargoes. Each tank is equipped with an individual cargo line, hatch access, deepwell pump, steam heating lines, and ventilation pipes. Although it appears cluttered, the deck space is devoted to a complex, well-marked, interconnecting piping system of noncorrosive pipes lined with stainless steel. The entire system is closed, and ventilation pipes are exhausted well above the heads of crewmen. On-board pumps are operated from a central control room which also houses remote gauges to monitor and maintain cargo temperatures up to 82°C.

Cargo can be taken on board or discharged at either the port or starboard sides at a rate of 250 tonnes per hour per pump. Easily accessible, gasket-sealed standard manifolds facilitate attachment to flexible pipes from shore establishments and minimize leakage. This modern vessel is well equipped with up-to-date navigational aids.

After discussing with the ship's officers the problems of loading, storing, and discharging cargoes, the panel climbed down into a 737-m^3 stainless steel tank. It then asked questions concerning the operations involved in cleaning such a structure to remove traces of the previous cargo.

Prior to entry by the ship's personnel, air samples are taken the top, middle, and bottom of the tank with Draeger tubes, which react chemically and produce a color reaction. After entry, air samples are drawn from the corners. Two landings connected by stairs from the access hatch are located on one side of the tank, providing platforms from which the walls can be flushed down by warm or hot water or steam under pressure. The operators wear protective clothing and a gas mask or a self-contained breathing apparatus. The residue is pumped out to a holding tank and/or is transferred to a special disposal barge when in port. While workers are inside the tank, there is always someone on deck who periodically checks to ensure that they have not been affected by any volatile residues.

Subsequently, the panel examined the safety equipment of the officers and crew, including protective clothing and self-contained respirator packs for short- (20-minute) and long-term use.

The panel belives that this vessel was far superior to many of the vessels and barges that carry volatile and potentially hazardous chemical cargoes. This 6-year-old ship met a high level of safety standards, having been built specifically for this purpose unlike many older ships which were neither originally built nor converted to meet present day safety standards. The senior officers of the

vessel were well aware of the dangerous and toxic nature of the cargoes carried. They had obtained such knowledge through courses held periodically by the company for their personnel. The panel was impressed by the responsibility with which the Stolt-Nielsen companies trained their officers, especially the executive officer who controls deck operations. It was obvious that few operations on this vessel would be conducted in a slipshod manner, a point confirmed by Lt. Cmdr. Robert Storch of the Houston Coast Guard inspectorate section. Unfortunately, time and opportunity did not permit the panel to visit other ships and barges to allow a comparison of safety features as well as loading and discharging features.

Stolt-Nielsen, Inc. requires an annual medical examination of its personnel. Reports of the results of these examinations are filed in the company's head office in Greenwich, Connecticut. The declaration of health forms pertain only to the physical examination and a chest X-ray. It does not include a blood chemistry or hematological assessment. The panel believes this to be a deficiency since such analyses might indicate alterations in organ function.

The impressive responsibility shown by Stolt-Nielsen, Inc. for its employees may not be the rule for many shipping firms; however, the panel has no comparative information with which to verify this.

APPENDIX B

MATHEMATICAL MODELS FOR CHEMICAL INTERACTIONS

A discussion of possible models for chemical interactions requires the review of some of the basic statistical concepts of bioassay.

PARALLEL LINE ASSAY AND RELATIVE POTENCY

Let the proportion response to chemical 1 at dose D_1 be denoted by $P_1(D_1)$ and to chemical 2 at dose D_2 be denoted by $P_2(D_2)$. Parallel line assay implies that $P_1(D) = P_2(fD)$ for all doses, D, where f is the relative potency of chemical 1 to chemical 2.

Example: Consider the "one hit" model, which is often appropriate to use in experiments with low doses of carcinogens and cell transformation:

$$P_1(D) = 1 - \exp(-\lambda_1 D),$$

$$P_2(D) = 1 - \exp(-\lambda_2 D),$$

where λ_1 and λ_2 are parameters (called "transformicities" by Gart et al., 1979) that are measures of the potency of the chemicals. For small doses, $P_1(D) \sim \lambda_1 D, P_2(D) \sim \lambda_2 D$, i.e., response is proportional to dose. When presented as a log-log plot, parallel straight lines are obtained:

$$\ln\{-\ln[1-P_1(D)]\} = \ln \lambda_1 + \ln D$$

$$\ln\{-\ln[1-P_2(D)]\} = \ln \lambda_2 + \ln D$$

The lines are parallel with slope 1. The relative potency is $f = \lambda_1/\lambda_2$, since

$$P_1(D) = 1-\exp[-(\lambda_1/\lambda_2)\lambda_2 D] = P_2(fD)$$

for all doses.

INDEPENDENT ACTION

Independent action may be defined by using the ordinary rules of probability in relation to the joint application of two doses, D_1 and D_2. If the probability of such a response is $P(D_1,D_2)$, then the independent action model implies:

$$P(D_1,D_2) = P(D_1,0) + P(0,D_2) - P(D_1,0)\ P(0,D_2).$$

Example: Using the "one hit" model again, we have:

$$P(D_1,D_2) = 1-\exp(-\lambda_1 D_1)+1-\exp(-\lambda_2 D_2)-[1-\exp(-\lambda_1 D_1)][1-\exp(-\lambda_2 D_2)] \text{ and}$$

$$P(D_1,D_2) = 1-\exp(-\lambda_1 D_1-\lambda_2 D_2). \quad (1)$$

Note that one possible dependent model is:

$$P(D_1,D_2) = 1-\exp(-\lambda_1 D_1 - \lambda_2 D_2 e^{\theta D_1}), \quad (2)$$

where, θ is a parameter which, when $\theta = 0$, measures the lack of independence of the model. Note that

$$P(D_1,0) = 1-\exp(-\lambda_1 D_1) \text{ and}$$

$$P(0,D_2) = 1-\exp(-\lambda_2 D_2).$$

That is, there is a parallel line assay, but independent action is not present.

DOSEWISE ADDITIVITY

If D_1' and D_2' individually yield the same response (e.g., 50%) then they are "dosewise" additive if the mixture consisting of $\pi D_1'$ and $(1-\pi)D_2'$ (for all $0<\pi<1$) leads to the same response (e.g., 50%) (Gaddum, 1953).

Example 1. Using the one hit curves and the independent model (Equation 1, above), the LD50's are $D_1' = (\ln 2)/\lambda_1$ and $D_2' = (\ln 2)/\lambda_2$. For all $0<\pi<1$, we find that $P[\pi D_1', (1-\pi)D_2'] = 1-\exp[-\lambda_1\pi(\ln 2)/\lambda_1 - \lambda_2(1-\pi)(\ln 2)/\lambda_2] = 1-\exp[-\ln 2] = 1/2$. This model is dosewise additive. Equation 2 will not generally enjoy this property. It is not necessary that all independent models be dosewise additive, but this one hit model (Equation 1) enjoys all three properties of parallelism, independence, and additivity.

Example 2. Gullino et al. (1956) used the concept of dosewise additivity in investigating the toxicity of essential amino acids in animals. In this effort, Cornfield (1975) served as statistical advisor. In an initial series of experiments, they determined the individual response curves of 10 such amino acids. More than 1,000 experiments would be required to determine all combinations. The investigators considered this to be impractical. Moreover, they believed that if the acids were dosewise additive, further such study would be unrewarding. They also studied pairs of acids at doses that yielded approximately 1% to 3%. A mixture under the dosewise additivity model should have yielded approximately this magnitude of response. However, 100% lethality was observed in all the pairwise combinations tested. When all 10 were combined at doses that should have yielded 50% response under the model, none died. From tests of all 10 combinations of 9 acids each, the investigators found that the one lacking L-arginine was less toxic than the rest. They attributed the protective effect of L-arginine to its ability to speed the metabolism of ammonia.

The concept of dosewise additivity can be quite useful in designing

a series of laboratory experiments.

MEASURES OF SYNERGISM AND ANTAGONISM

Much of the discussion of deviation from independence concerns its epidemiological applications (see, for example, Hamilton and Hoel, 1978; Walter, 1976). Such applications are typically defined in terms of the relative risk (R) of exposed individuals [P(D)] to unexposed individuals [P(0)], i.e., $R = P(D)/P(0)$. Unexposed individuals are assumed to have a background risk (α).

The modification of the one hit curves is as follows:

$$P(D_1,D_2) = \alpha + (1-\alpha)[1-\exp(-\lambda_1 D_1-\lambda_2 D_2)],$$

$$P(D_1,0) = \alpha + (1-\alpha)[1-\exp(-\lambda_1 D_1)], \text{ and}$$

$$P(0,D_2) = \alpha + (1-\alpha)[1-\exp(-\lambda_2 D_2)].$$

The relative risks to zero doses are:

$$R(D_1,D_2) = 1 + \frac{(1-\alpha)}{\alpha}[1-\exp(-\lambda_1 D_1-\lambda_2 D_2)], \tag{3}$$

$$R(D_1,0) = 1 + \frac{(1-\alpha)}{\alpha}[1-\exp(-\lambda_1 D_1)], \text{ and} \tag{4}$$

$$R(0,D_2) = 1 + \frac{(1-\alpha)}{\alpha}[1-\exp(-\lambda_2 D_2)]. \tag{5}$$

Various measures of synergism or antagonism have been proposed (see, for example, Hamilton and Hoel, 1978). Specific proposals of Hogan et al. (1978), Finney (1971), and Walter (1976) are designated below as S_H, S_F, and S_W, respectively:

$$S_H = R(D_1,D_2) - R(D_1,0) - R(0,D_2) + 2$$

$$S_F = \frac{R(D_1,D_2) - 1}{R(D_1,0) + R(0,D_2) - 2}$$

$$S_W = \frac{R(D_1,D_2)}{R(D_1,0)R(0,D_2)}$$

In all three examples, independence is indicated by S = 1, synergism by S > 1, and antagonism by S < 1. Risks in the first two are essentially additive, whereas in Walter's proposal, they are multiplicative.

For the independent action one hit model and for small doses, (e.g., $R(D_1,D_2) \sim 1+[(1-\alpha)/\alpha][\lambda_1 D_1 + \lambda_2 D_2)]$, all three equations yield approximately 1:

$$S_H \sim 1+\frac{(1-\alpha)}{\alpha}[\lambda_1 D_1+\lambda_2 D_2 - \lambda_1 D_1-\lambda_2 D_2] = 1,$$

$$S_F \sim \frac{[(1-\alpha)/\alpha][\lambda_1 D_1+\lambda_2 D_2]}{[(1-\alpha)/\alpha][\lambda_1 D_1+\lambda_2 D_2]} = 1, \text{ and}$$

$$S_W \sim \frac{1 + [(1-\alpha)/\alpha](\lambda_1 D_1+\lambda_2 D_2)}{\{1 + [(1-\alpha)/\alpha]\lambda_1 D_1\}\ \{1+[(1-\alpha)/\alpha]\lambda_2 D_2\}} \sim 1.$$

This means that for a small absolute probability of response all these measures will yield approximately 1, indicating independence. In many situations, e.g., when smoking is associated with lung cancer, a low probability is reasonable although the relative risk may be high. This does not mean that they shall yield similar values under other circumstances.

Example 1: Consider the effect of smoking and asbestos on lung cancer, which was investigated by Hammond and Selikoff (1973). Let D_1 refer to smoking and D_2 to asbestos:

$$R(D_1,0) = 11$$

$$R(0,D_2) = 2$$

$$R(D_1,D_2) = 90$$

$$S_H = 90 - 11 - 2 + 2 = 79$$

$$S_F = \frac{90 - 1}{11 + 2 - 2} = 8.1$$

$$S_W = \frac{90}{11 \times 2} = 4.1$$

Thus, although all three measures show synergism, their magnitude can vary widely.

Example 2: Consider the effect of cigarette smoking, D_1, and exposure to radon daughters, D_2 (Lundin et al., 1969):

$$R(D_1,0) = 11$$

$$R(0,D_2) = 4$$

$$R(D_1,D_2) = 41$$

$$S_H = 41 - 4 - 11 + 2 = 28$$

$$S_F = \frac{41 - 1}{11 + 4 - 2} = 3.1$$

$$S_W = \frac{41}{4 \times 11} = 0.9$$

Although S_H and S_F indicate synergism, S_W does not. Clearly "additive" but not "multiplicative" synergism is present.

There is no consensus concerning the measure of synergism that is appropriate (Rothman, 1978a,b; Walter and Holford, 1978). Still other measures may also be proposed.

MATHEMATICAL JUSTIFICATION OF THE "THRESHOLD LIMIT" CRITERION [$\Sigma(C_i/T_i)>1$] BY THE INDEPENDENT ONE-HIT MODEL

The "Threshold Limit Document" (American Conference of Governmental Hygienists, 1977, p. 45 ff.) considers a criterion based on the quantity $\Sigma(C_i/T_i)$, where C_i denotes the observed concentrations and T_i the corresponding threshold limits for a series of chemicals $i = 1,2,\ldots n$. If $\Sigma(C_i/T_i)$ exceeds unity, then the threshold limit of the mixture is considered to be exceeded. This criterion may be justified under the assumption of a generalized independent one hit model:

$$P(D_1,\ldots D_n) = 1-\exp(-\lambda_1 D_1 -\ldots\lambda_n D_n).$$

Let P_o be an "acceptable probability" of an adverse response. Considering each individual chemical exposure only, this P_o may be used to define the threshold value:

$$P_o = P_i(T_i) = P(0,\ldots 0,\ T_i,0,\ldots 0) = 1-\exp(-\lambda_i T_i).$$

For small P_o, which is of course reasonable:

$$P_o \sim \lambda_i\ T_i, \text{ and thus,}$$

$$\lambda_i \sim P_o/T_i.$$

Conversely, for each small dose below the threshold,

$$P_i(C_i) \sim \lambda_i\ C_i \leq P_o, \qquad i = 1,2,\ldots n.$$

Substitution for λ_i yields the approximate relation, $C_i/T_i \leq 1$, for $i = 1,2,\ldots n$, which is the single chemical threshold.

Now require that the risk of exposure to n chemicals at doses $C_1, C_2, \ldots C_n$ be less than the acceptable probability, P_o. Under the independent one hit model for small doses,

$$P(C_1, C_2, \ldots C_n) \sim \Sigma (\lambda_i C_i) \leq P_o,$$

since $\lambda_i = P_o/T_i$. This may be written:

$$P_o \Sigma(C_i/T_i) \leq P_o, \text{ or}$$

$$\Sigma(C_i/T_i) \leq 1.$$

This is the same criterion that was cited by the American Conference of Governmental Industrial Hygienists (ACGIH) (1977), but it has a different meaning. The probability model assumes a positive, but small, probability of response below T_i. Thus, it is possible that the n chemicals could affect different organs, but their accumulative probability of an adverse response at one or more organs will exceed P_o whenever $\Sigma(C_i/T_i) > 1$. The ACGIH does not use the additive criterion when the "harmful substances are not in fact additive, but <u>independent</u> as when purely local effects of different organs of the body are produced by the various components of the mixture." The ACGIH criterion assumes a perfect threshold, i.e., absolutely no adverse response below T_i for each T_i. Thus, this definition of "independent" and "additive" is very different from the sense in which these terms are used in the probability arguments advanced here.

When synergism may exist between two chemicals and the following model for low doses is assumed (see Equation 2, above):

$$P(D_1,D_2) = \lambda_1 D_1 + \lambda_2 D_2 \exp(\theta D_1).$$

It is easily seen that

$$T_1 = P_o/\lambda_1 \text{ and } T_2 = P_o/\lambda_2).$$

From this we may assume:

$$P(C_1,C_2) = \left(\frac{C_1}{T_1} + \frac{C_2}{T_2} \exp(\theta C_1)\right) P_o$$

Clearly, even if $C_1/T_1 + C_2/T_2 \leq 1$, it is possible when θC_1 is large enough for $P(C_1,C_2)$ to exceed P_o. Thus, in the presence of synergism this criterion can fail to provide suitable protection.

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