SUPPLEMENTAL MATERIAL, APPENDIX 1 – Types of Environmental Mixtures

Table 1-1. Examples of 'Similar' Mixtures Composed of Agents that have Comparable Properties (mixture composition can change significantly over the time period of interest).

Similar Chemical Structures and/or Properties	
Aldehydes	Phthalates
Carbamate Pesticides	Phytoestrogens
Dioxins	Polybrominated Diphenyl Ethers
Furans	Polychlorinated Biphenyls
Heavy Metals	Polycyclic Aromatic Hydrocarbons
Organophosphate Pesticides	Trihalomethanes
Organochlorine Pesticides	Volatile Organic Compounds

Similar Toxicological Properties and/or Effects	
Allergic Contact Sensitizers	Hepatotoxicants
Genetic Carcinogens	Immunotoxicants
Nongenetic Carcinogens	Nephrotoxicants
Cardiovascular Toxicants	Neurotoxicants
Cholinesterase Inhibitors	Respiratory Toxicants
Eye Irritants/Toxicants	Reproductive Toxicants
Hematotoxicants	Skin Irritants/Toxicants
Hormonally Active Agents	Teratogens/Developmental Toxicants

Table 1-2. Examples of 'Defined' Mixtures that are Created at a Given Time and Place, and that have a Reasonably Defined Composition, at Least Initially (mixture components do not necessarily have similar properties).

Agricultural Runoff	Disinfection By-Products
Gasoline-Powered-Engine Emissions	Environmental Tobacco Smoke
Cigarette Smoke	Hazardous Waste Site Constituents
Coal-fired Power Plant Emissions	Photochemical Smog
Coke Oven Emissions	Petroleum Refinery Emissions
Diesel-Powered-Engine Emissions	Wood-Burning Emissions

Table 1-3. Examples of 'Coincidental' Mixtures that Occur by Happenstance at a Time and Place of Interest (mixture constituents do not necessarily have similar properties, the composition is not necessarily constant, and the mixture may occur frequently, occasionally, or rarely).

Breast Milk Contaminants	Residential Exposures
Dietary Agents	Soil Contaminants
Dust Contaminants	Stressors in Poor, Inner-City Neighborhoods
Indoor Airborne Contaminants	Urban Air Pollution
Occupational Exposures	Waterborne Agents

Table 1-4. Categories of Potentially Hazardous Agents that can Contribute to Mixtures Encountered in Occupational (Industrial) Environments (adapted from Tarcher 1992).

Aerosols, Vapors, Gases (a)	
carbon monoxide	phosgene
formaldehyde	smoke
hydrogen sulfide	sewer gas
ethylene oxide	sulfur dioxide
nitrogen oxide	inert gases
ozone	welding fume
diesel exhaust	combustion byproducts
Biological Inhalants (b)	
bacteria	fungi
molds	spores
Corrosive Substances (c)	
acids	alkalis
ammonia	chlorine
phenol	
Dyes, Stains (d)	
aniline dyes	azo dyes
benzidine	
Explosive Components (e)	
nitro-organics (e.g., TNT)	
perchlorate and other oxicants	
Inorganic Dusts, Powders (f)	
asbestos	beryllium
coal dust	fiberglass
talc	silica
Insecticides, Herbicides (g)	
carbamates	halogenated HCs
organophosphates	phenoxyherbicides
conazoles	
Ionizing/Nonionizing Radiation (h)	
X ray	UV radiation
EM radiation	
Metals, Metal Fumes (i)	
aluminum	iron
arsenic	lead
cadmium	mercury
chromium	nickel
cobalt	
Organic Dust (j)	
cotton dust	wood dust
poison oak	
Petrochemicals (k)	
asphalt	tar
creosote	coal tar
PBBs, PCBs	petroleum distillates
Physical Agents (l)	
heavy lifting	noise
thermal stress	vibration
Plastics (m)	
vinyl chloride	epoxy resins
acrylonitrile	polystyrene
Sensitizing Agents (n)	
aliphatic amines	nickel
toluene diisocyanate	platinum
hexavalent chromium	
Solvents (o)	
benzene	carbon disulfide
carbon tetrachloride	chloroform
methanol	perchloroethylene
trichloroethylene	xylene
glycol ethers	toluene

EM = electromagnetic; HC = hydrocarbons; PBBs = polybrominated biphenyls; PCBs = polychlorinated biphenyls

Table 1-5. Examples of Potential Exposures to Mixtures by Occupation (Adapted from Tarcher 1992).

Occupation or Activity	Likely Mixture Constituents[a]
Agriculture and Pest Control	a,b,f,l
Automobile/Aircraft Manufacturing & Repair	a,c,f,i,l,n
Bakers, Food Handlers	b,m,n
Boiler Operations & Cleaning	a,c,f,l
Building Maintenance, & Plastering	c,d,f,l,k,o
Ceramics & Masonry	f,i
Carpentry, Woodworking, & Lumber Industry	b,j,k,l,o
Chemical Industry and End Uses	a,o
Construction, Demolition, and Road Work	c,d,e,f,l,k.o
Dry Cleaning & Laundry	k,n,o
Electric Work, Electronics	c,f,i,k,n
Firefighters	a,e,l
Foundry Work	a,c,f,i,l
Health Care, Laboratory Work, & Dental Work	a,b,c,d,f,h,l,m,n,o
Machinery Work, Grinding, & Metal Work	a,c,i,l,n,o
Mining (particularly underground)	a,f,h,l
Oil & Petrochemical Work	a,c,k,l,o
Paper Industry	f,o
Plastic & Plastic Product Manufacturing	f,k,m
Plumbing, Pipefitting, & Shipfitting	a,c,f,i,l
Printing & Lithography	d,j,l,o
Sandblasting & Spray Painting	a,f,i,l,o
Shipyard & Dock Work, Transportation	a,c,f,i,k,l,o
Textile Industry	a,d,f,j,o
Welding	a,f,i,n

[a]See Table 4 for explanation of mixture constituents

Table 1-6. Categories of Agents that can Contribute to Mixtures Inside Residences.

Aerosols, Vapors, Gases
- VOCs (e.g., benzene, styrene, toluene, formaldehyde, chloroform, xylenes)
- cooking & heating fuel combustion by-products (e.g., CO, CO_2, NO_2, BAP)
- environmental tobacco smoke (contains thousands of individual compounds)
- infiltration of outdoor air pollution (e.g., automotive and industrial emissions)
- reintrainment of house dust (e.g., biological and chemical composition of aerosols)

Biological Inhalants (aeroallergens and aeropathogens)
- insects and other arthropods, including excretions and body fragments (e.g., mites, cockroaches)
- bacteria (e.g., staphylococcus aureus, salmonella typhosa)
- fungi (e.g., aspergillus niger, penicillium funiculosum)
- pet dander (e.g., cats, dogs, hamsters, rabbits)
- yeasts (e.g., saccharomyces cerevisae, candida albicans)

Dusts, Fibers
- asbestos fibers
- fiberglass fibers
- mineral wool fibers
- house dust

Insecticides, Fungicides, Herbicides
- carbamates
- organophosphates
- halogenated hydrocarbons
- phenoxyherbicides

Ionizing/Nonionizine Radiation
- radon and radon progeny
- electromagnetic radiation

Psychosocial Stressors
- family issues
- crowding
- poverty

Metals (particle-bound)
- arsenic
- lead
- cadmium
- chromium

Physical Agents
- noise
- lighting
- thermal stress
- ergonomics
- humidity

Odors
- cooking
- consumer products
- pets
- people
- tobacco
- trash

BAP = benzo(a)pyrene, CO = carbon monoxide, CO_2 = carbon dioxide, NO_2 = nitrogen dioxide, VOCs = volatile organic compounds

Table 1-7. Categories of Conditions/Sources that can Contribute to Mixtures Encountered by Residents in Economically Disadvantaged Inner-City Communities.

Basic Sanitation	Built Environment
unavailability of drinking water	lack of green space
unsafe drinking water	lack of walking venues
inadequate trash collection/disposal	substandard housing
insufficient sewage handling/treatment	abandoned property
rodent infestation	poor road conditions
inadequate drainage	locally unwanted land uses
insufficient animal control	lack of urban amenities
Environmental Pollution	Neighborhood Quality
automotive and industrial air pollution	residential crowding
indoor air pollution	truck and train traffic
industrial and municipal water pollution	traffic congestion
contaminated soil/dust	crime/violence
hazardous waste sites	street noise
contaminated gardens	noxious odors
contaminated locally caught fish	low property values
Lifestyle Choices	Personal and Family Issues
alcohol use	un- or underemployment
diet/nutrition	hazardous work
fitness/physical activity	poverty
personal hygiene	lack of insurance
illicit drug use	limited access to health care
sexual behavior	language problems
tobacco use	family conflict
	lack of emotional support

SUPPLEMENTAL MATERIAL, APPENDIX 2 – Cumulative Exposure and Increased Vulnerability

Rhomberg (1999) proposed a simplified conceptual scheme for describing the cumulative toxicologic impact of individual exposure-time profiles for stressors that act in an additive manner. Assume that an individual's exposure history for chemical A over the relevant time period is reconstructed by monitoring, modeling, or use of scenario approaches (See Figure 2-1, part a). Also assume that the body burden (internal dose) of the chemical at any given point in time is a function of the dose at that time point and whatever chemical (or metabolite, reaction product, or cumulative damage marker) that remains from previous exposures. Further assume that the contribution of any particular exposure to the body burden decreases with time as a function of the chemical's half-life in the body (See Figure 2-1, part b) or the rate of reversal of causal intermediates along the toxicologic pathway to end effects (e.g. cholinesterase inhibition at relevant places in the brain or peripheral nervous system). Application of this half-life discounting function to an individual's exposure history produces a kind of "moving average" body burden, where the contribution of specific exposures are weighted by their temporal proximity to each time point. As shown in Figure 2-2, the result is a continuous estimate of the cumulative body burden of a particular chemical or causal intermediate over an individual's exposure history (Mileson et al. 1999, Rhomberg 1999).

Now consider that the hypothetical individual discussed above is exposed concurrently over time to two chemicals, A and B, that have a common mechanism of toxicity, which is to say that the chemicals cause the same toxic effect in or at the same

organ or tissue by essentially the same series of biochemical events -- oganophosphate insecticides, for example. Organophosphate insecticides act by irreversibly inhibiting acetyl cholinesterase enzyme molecules. These enzyme molecules normally inactivate the chemical (acetylcholine) involved in neuromuscular signaling after it has been sent and received. The inhibition caused by the organophosphate insecticides is thought to be reversed as the neurons regenerate new acetyl cholinesterase molecules over a period of weeks. Similar acetyl cholinesterase signaling also occurs between neurons in both the brain and the peripheral nervous system.

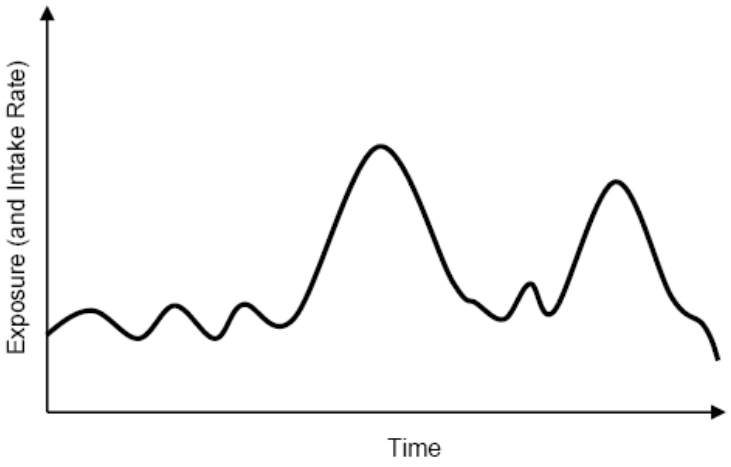
As shown in Figure 2-2, part a, the time profile of body burden for both chemicals A and B is reconstructed as described earlier. Because the two chemicals have the same mechanism of action, they are presumed to be affecting the same organ or tissue. Consequently, the total body burden is a function of the combined load of both chemicals. Assuming that the total toxicologic burden is additive – the sum of the dose of chemical A plus chemical B expressed in equivalent units – it can be represented by the joint burden depicted in Figure 2-2, part b. The joint toxicologic burden is an approximation of the cumulative load on the target tissue as it varies over time from concurrent exposure to chemicals A and B (Mileson et al. 1999, Rhomberg 1999).

As Rhomberg (1999) points out, this approach provides a means to gauge the probability of harm from the cumulative toxicologic burden of current and past (weighted by their continuing effect on the present) exposures to two or more stressors by comparison with either short-term (e.g., days, weeks) or long-term (e.g., years, decades) health-related benchmarks (see Figure 2-2, part b). Furthermore, this

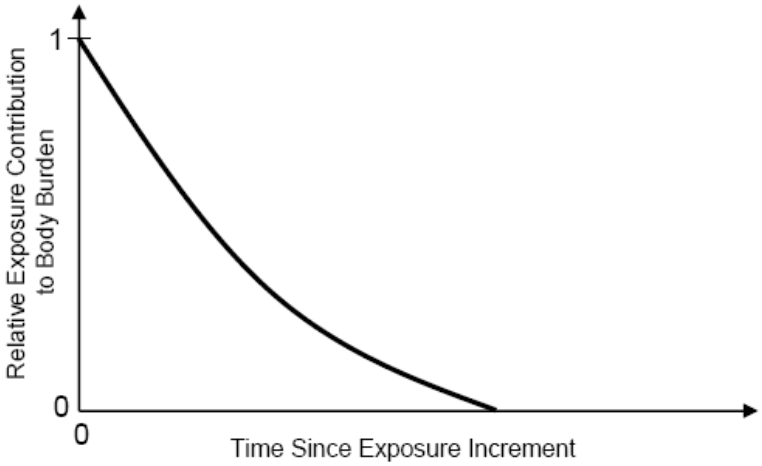
conceptual framework can be refined and expanded as more detailed exposure and toxicological information becomes available.

It should be obvious by now that assessing cumulative exposure to multiple chemicals is complicated. But the reality can be even more complex than the situations and conditions portrayed in Figures 2-1 and 2-2, wherein doses and responses are assumed to be additive, effects are assumed to be dependent on total dose (body burden) independent of the pattern (timing) of exposures, and mixture constituents are assumed to act via a shared toxicologic mechanism. We know that these simplifying assumptions are not valid in many cases because some exposures produce more than additive effects (e.g., tobacco smoke and asbestos), exposure sequence (e.g., prior exposure to one toxicant causes increased susceptibility to another toxicant) and timing (e.g., chemical exposure during fifth and sixth weeks of fetal development, when sexual differentiation occurs, can affect the development, growth, and functioning of the reproductive system) are critical in certain cases, and mixture-related effects can occur through a combination of toxicity modes (e.g., dioxin-like substances can cause both physiologic changes and direct cell damage) (Carpenter et al. 2002). Yet despite the knowledge gaps and deficits of scientific understanding, exposure assessors are confronted with the need to evaluate how differential cumulative exposure affects vulnerability.

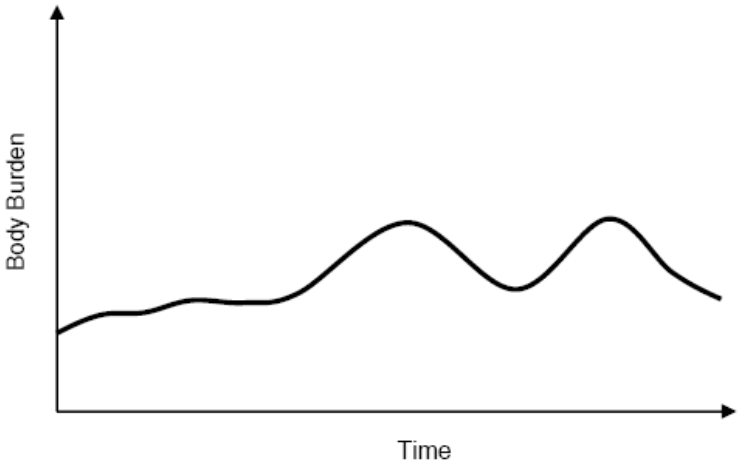
List of Figures for Appendix 2



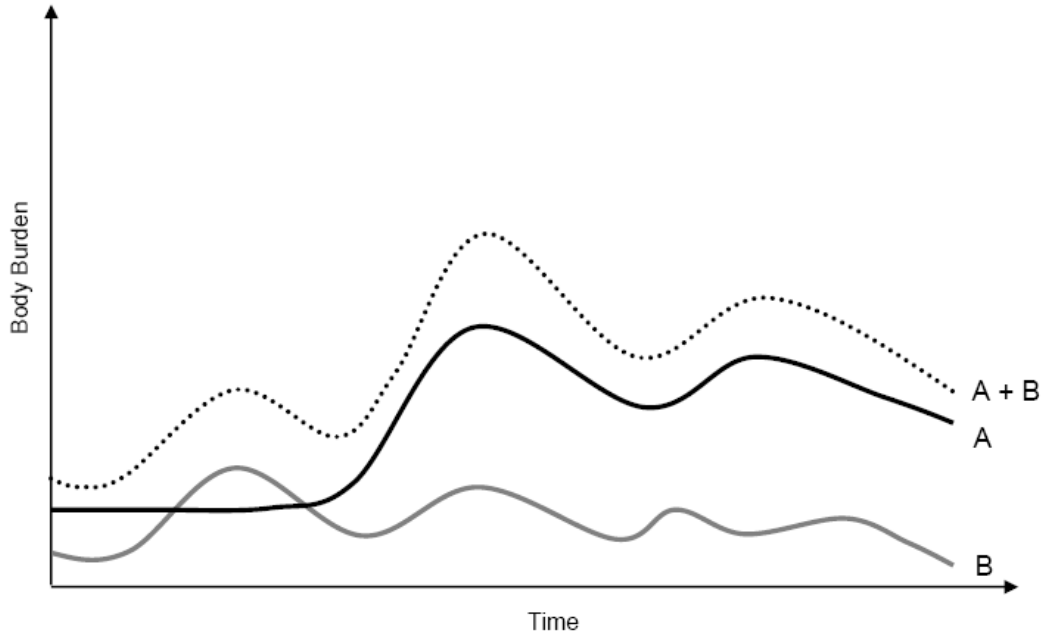
A. Exposure History



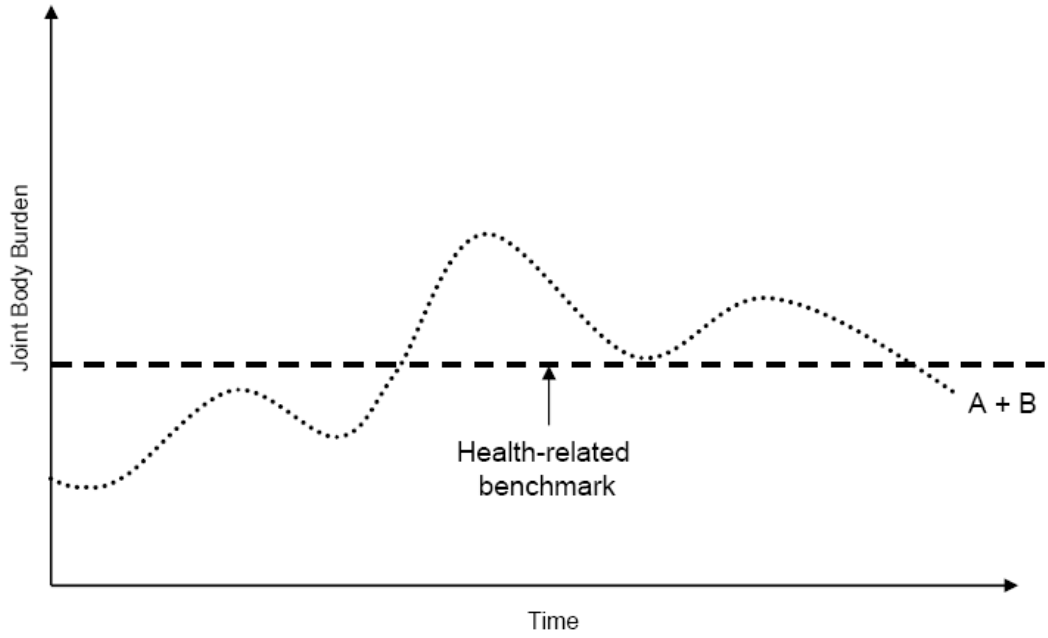
B. Exposure Contribution to Body Burden



C. Body Burden Over Time



A. Joint Burden Derived by Additivity



B. Joint Burden vs. Benchmark

SUPPLEMENTAL MATERIAL, APPENDIX 3 – Physiologically-Based Pharmacokinetic Modeling

The last two decades have seen a flowering of the use of physiologically-based pharmacokinetic (PBPK) models that form the logical basis for mathematical analyses of pharmacokinetic interactions. PBPK models form a natural focus for integration of the combined dose of a single chemical absorbed by multiple routes, exposures to multiple chemicals that have metabolic relationships (Barton et al., 2000), or multiple chemicals that affect and are acted upon by the same enzyme systems. At the molecular level, the basic Michaelis-Menten model of enzyme kinetics provides a framework for quantitative modeling of various types of enzyme (and transport) inhibition effects. However, in general, there is only the crudest understanding of quantitative dose-time-response relationships for the induction of changes in the activity of either metabolizing enzymes, active transport systems or key co-factors (such as the sulfhydryl-containing tripeptide, glutathione) that are often required for specific enzyme-catalyzed reactions.

Pharmacokinetic models describe the uptake, transport, chemical transformation, and elimination of substances from the body. There are two contrasting traditions of pharmacokinetic modeling: classical pharmacokinetic modeling and physiologically based pharmacokinetic modeling. Classical pharmacokinetic modeling seeks the simplest possible mathematical form to describe the changes over time in the observable amount of a substance (usually a drug) in an accessible fluid (usually blood, or some blood component such as serum). “Compartments” in this framework need not refer to specific physical or chemical “locations,” but are created for mathematical

convenience in describing data sets, usually using exponential equations with one or more terms of the form:

$$C_{blood} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + \ldots$$

In this equation, C_{blood} is the concentration of the chemical in the measured location, t is time, and the A's represent the initial effective concentration in various subcompartments of the body that exchange material with the central compartments with rate constants defined as k_1, k_2, … etc. Most of the available in vivo pharmacokinetic measurements from the pharmaceutical literature (e.g. clearances, half lives, volumes of distribution) derive from this classical pharmacokinetic modeling tradition. However, because there is no natural way to incorporate interaction mechanisms in this modeling framework, we will not discuss it further.

Physiologically-based pharmacokinetic (PBPK) or, for toxicants, toxicokinetic (PBTK) modeling seeks a more mechanistic description of physiological systems that incorporates data on the physical sizes of organs or groups of organs, blood flow rates, and other measurements. Thus it draws on a wider variety of information than the simple observation of concentration over time in a specific body location. All these data are integrated into a coherent picture intended to represent real processes, albeit with some necessary simplification compared to the actual biological system. Bond and Medinsky (1995) provide examples of the application of this framework for quantitative analysis of interactions between chemicals metabolized by the same enzyme systems.

As shown in Figure 3-1, PBTK models of simple volatile halogenated organics typically break the process down into several "compartments," which are grouped according to the ratios of blood flows to tissue volumes. The "boxes" in this figure

represent integrals over time of the processes of uptake and release of toxicant from each compartment. The rate of release of the toxicant with blood flowing out from each compartment directly depends on the blood flow rate divided by the product of the tissue volume and the tissue/blood partition coefficient (the latter being usually measured in separate in vitro experiments). [Not shown in this diagram is an assumed equilibration between alveolar air and the blood flowing through the aleveoli, allowing uptake or exhalation of the volatile organic.]

From the standpoint of interaction analysis, the aspect of this kind of model system that is most frequently important is the process of metabolism of the toxicant in the liver. Although metabolic processing can also occur in other organs, PBTK modelers by convention usually place it all in the liver unless there is good reason to do otherwise.

Metabolism is accomplished by a small number of large enzyme molecules that greatly speed up select chemical reactions by providing active sites that bind the "substrates" (reacting chemicals) and stabilize "activated" forms of the chemicals that serve as intermediates between the reactants and the products. When there is only a single substrate being converted into a product, the rate of production of the product P is usually governed by the Michaelis-Menten equation:

$$\text{Rate of Production of P (product)} = \frac{V_{max}[C]}{K_m + [C]}$$

where V_{max} is the maximum rate of production of P that can occur at the limit of high concentration of the substrate C, and K_m is usually known as the Michaelis Constant,

which is defined as the substrate concentration that elicits half of the maximum rate of production of the product. At a very high concentration of the substrate (when [C] is many times K_m*), the reaction approaches a maximum because the limited number of large enzyme molecules are working as fast as they can; as soon as one molecule of the substrate is finished being converted into product, another molecule of substrate is immediately available to diffuse in to the active site to replace it. At the limit of low doses (where the substrate concentration [C] is much lower than K_m) the reaction proceeds nearly linearly with a rate constant equal to the ratio of V_{max}/K_m. The reason for the approach to low dose linearity is that at low substrate concentrations the rate of the reaction is limited by the rate at which molecules of the substrate randomly collide with and bind to the active site of the enzyme. This collision rate, in turn directly depends on the number of free enzyme molecules and the number of substrate molecules per unit volume, multiplied by some factor dependent on the temperature. At very low concentrations, where enzyme molecules are essentially all in an unbound state, and the temperature is constant, this means there must be a linear dependency on substrate concentration.

Similar to enzymes, some specialized proteins in membranes are designed to speed up the transport of selected molecules across biological membranes. Some of these active transport proteins are found in the gut—transporting, for example calcium and other needed nutrients (along with some chemically similar toxicants, such as lead--Heaney et al., 1990); others serve the special needs of the fetus (Sibley and Boyd, 1992); and still others facilitate or reduce (via active energy-consuming “resorption”)

excessive excretion of needed salt ions via the kidney (van Ginneken and Russell, 1989.) In general, where kinetics are described quantitatively, these are usually modeled using the same equation form as is used for saturable Michaelis-Menten enzyme kinetics. In the case of the kidney, experiments with drugs have established competitive interactions between anions such as carboxylic acids, but not cations (positively charged ions) (Somogyi, 1987).

If two substrates bind to the same active site on the same enzyme such that binding of one substrate prevents the binding of the other, the two substrates are said to be "competitive" inhibitors. In the context of the Michaelis-Menten enzyme kinetic equation, the effect of this competition is to increase the K_m by an amount that depends on the relative binding affinity to the active site and the concentration of the inhibiting/competing substrate:

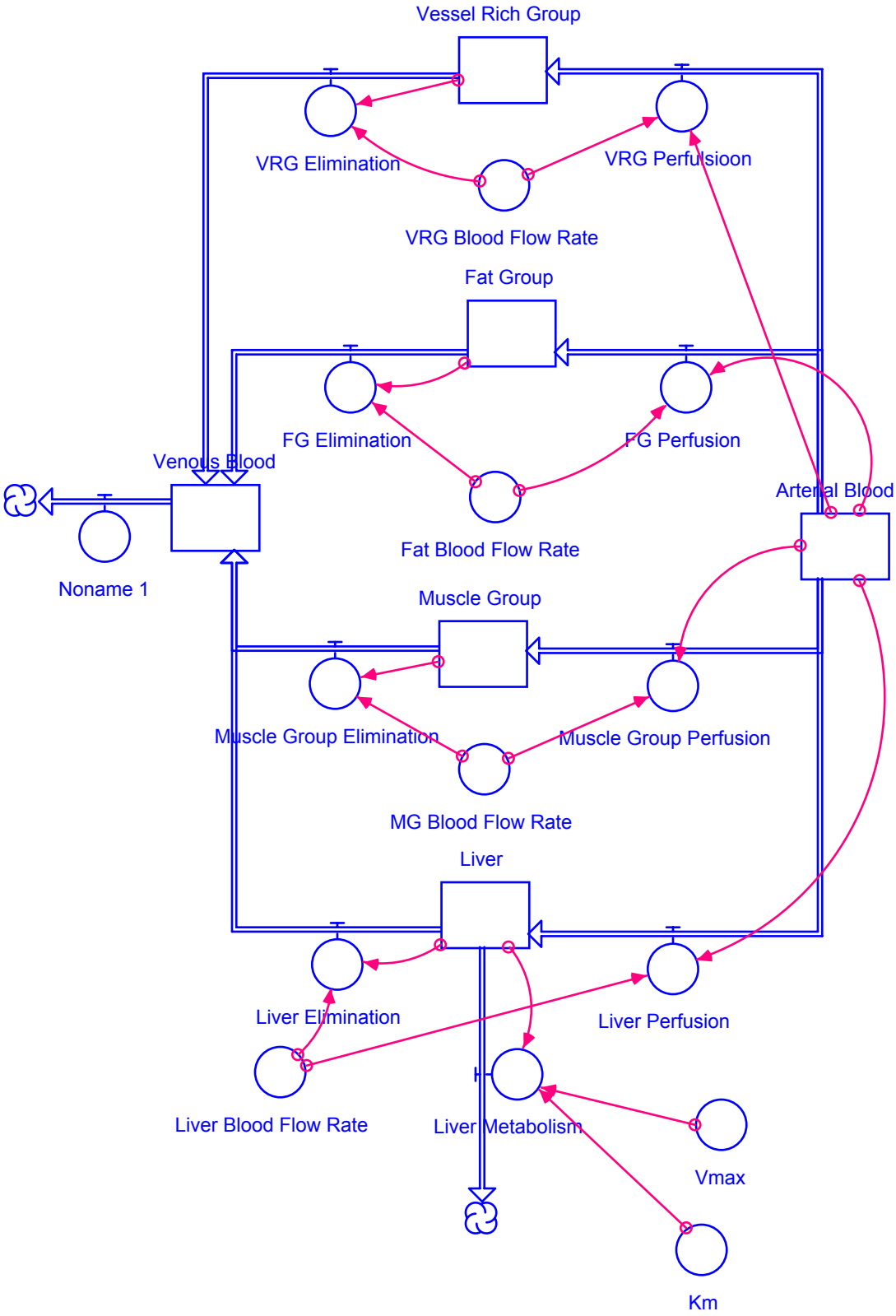
$$\text{Rate of Production of P (product)} = \frac{V_{max}[C]}{K_m\{1+[\text{Inhib}]/K_i\} + [C]}$$

Where [Inhib] is the concentration of the competitive inhibitor in the cells where the metabolizing enzyme is found, and K_i is the concentration of inhibitor that would be needed to increase K_m for the substrate processed into product P by two-fold (Krishnan et al., 1994; Yu et al., 2002). The effects of multiple competitive inhibitiors can be similarly modeled by adding additional terms in the form [Inhibitor2]/K_{i2} to the bracketed expression in the denominator (Krishnan et al. 2002).

Two other types of interactions for both enzymatic metabolism and transport (noncompetitive and uncompetitive) are discussed in Appendix 4.

There is an important set of exceptions to this picture of simple Michaelis-Menten kinetics. A well-known case that produces more complex behavior at moderate doses is the facilitated transport of oxygen by hemoglobin. The hemoglobin molecule has four binding sites, and oxygen binding at one site increases the affinity of the other sites for oxygen. The change continues with increasing numbers of oxygen molecules bound, so that the affinity of hemoglobin for the fourth oxygen molecule is about 300 times greater than its affinity for the first molecule (Rawn, 1983). The consequence of this for transport is that hemoglobin tends to go from an all-oxygen-free to an all-oxygen-bound state over a much narrower range of oxygen concentration than would be the case if the binding to different sites were independent--thus helping maintain relatively constant oxygen availability in the tissues over a relatively large range of rates of blood flow and tissue demand. Dimeric and polymeric receptors may similarly steepen the dose-effect relationships for many receptor-mediated signaling processes.

Figure 3-1
Diagram for a Physiologically-Based Pharmacokinetic Model for a Volatile Organic (Perchloroethylene—adapted from Hattis et al., 1993)


Vessel Rich Group
VRG Elimination
VRG Perfulsioon
VRG Blood Flow Rate
Fat Group
FG Elimination
FG Perfusion
Venous Blood
Arterial Blood
Fat Blood Flow Rate
Noname 1
Muscle Group
Muscle Group Elimination
Muscle Group Perfusion
MG Blood Flow Rate
Liver
Liver Elimination
Liver Perfusion
Liver Blood Flow Rate
Liver Metabolism
Vmax
Km

SUPPLEMENTAL MATERIAL, APPENDIX 4 – Noncompetitive and Uncompetitive Inhibition in the Framework of Michaelis-Menten Enzyme/Transport Kinetics

Sometimes an inhibitor can bind to the enzyme after its active site has already been occupied by the substrate, and still prevent conversion of the bound substrate to the product. This is termed "uncompetitive inhibition" (Krishnan et al., 1994; Yu et al., 2002). Quantitatively this results in modification of both the Vmax term in the numerator and the K_m term in the denominator:

$$\text{Rate of Production of P (product)} = \frac{[C]\dfrac{V_{max}}{1 + [Inhib]/K_i}}{[C] + \dfrac{K_m}{1 + [Inhib]/K_i}}$$

Finally, there is a category of "noncompetitive inhibition" in which the inhibitor binds to both the free enzyme (as in competitive inhibition) and also to the enzyme-substrate complex, inhibiting the production of product in both cases. These other modes of enzyme inhibition have not, to our knowledge, been widely reported in the modification of metabolic activities important to the processing of toxicants.

SUPPLEMENTAL MATERIAL, APPENDIX 5 – Immune-Mediated Drug-Induced Liver Diseases

Liu and Kaplowitz (2002) review the more specific case of immune-mediated drug-induced liver diseases. Among the hypothesized pathogenic mechanisms is the possibility that specific drugs or their reactive metabolizes can chemically modify specific host proteins—forming "haptens" that can be the sites of initial misidentification of the host proteins as "foreign". In some cases this is thought to result in damaging attack by the immune system on normal host liver constituents. Wulferink et al. (2001) provide evidence for this type of explanation in exploring mechanisms of the Spanish Toxic Oil Syndrome--observed following ingestion of rape seed oil contaminated with aniline in a tragic episode of poisoning of over 20,000 people (Tabuenca, 1981). Wulferink find that aniline itself and its non-protein-reactive metabolites nitrobenzene, p-aminophenol and N-acetyl-p-aminophenol, failed to elicit detectable immune responses. However aniline's reactive metabolites nitrosobenzene and N-hydroxylaniline did elicit immune responses, as did various lipid reaction products with aniline derivatives that were previously implicated in the Toxic Oil Syndrome. Other examples include autoimmune responses of some mice to dichloroacetyl chloride (produced from TCE by the high temperature welding) (Khan et al., 1997) and observations of Stejskal et al. (1999) of lymphocytes reactive to nickel, mercury and gold in some people. An interesting aspect of the Khan et al (1997) observations is a relatively prolonged time course—peak response were observed only following 4-8 weeks of treatments repeated every 4 days.

SUPPLEMENTAL MATERIAL, APPENDIX 6 – Methods for Cumulative Risk Assessment

Given the intrinsic challenges, which constrain the assessment of differential cumulative exposure and hamper the appraisal of related interactive effects, how should risk assessors go about evaluating the potential harm caused by mixtures of environmental stressors? The glib answer, of course, is that they should do so "very carefully," which is true but unhelpful. The reality is that risk assessors in the near term are going to have to make do with the data and methods on hand.

A comparison of existing methods for cumulative risk assessment, including assumptions, resource requirements, and strengths and weaknesses, is provided in Table 6-1 (Hertzberg and Teuschler 2002, Mileson et al. 1999, Purchase 2000, USEPA 2000). Ideally, it is preferable to use biologically-based physiologic, toxicokinetic and toxicodynamic models, but these are not currently on hand for most mixtures of concern. Moreover, their eventual development depends on application of considerable resources, which are not available in most cases. The next most preferable method is the interactive Hazard Index (HI_{INT}) approach, which modifies the Hazard Index according to evidence on pairwise interactions using a specified function (*f*) to describe empirical data for the combined effects of pairs of components. Next in preference is the more standard Toxicity Equivalency Factor (TEF) approach, which represents the toxicity of individual stressors relative to the potency of a reference stressor, followed by the margin of exposure (MOE) approach, which uses Toxicity Equivalency Factors to calculate the margin between the RfD or RfC and the estimated exposure. When none of these methods is applicable, then it is necessary to use either the Hazard Index

approach employing the no-observed-adverse-effect-level (NOAEL) or the Benchmark Dose (BMD_x) or, as a last resort, the Hazard Index approach using RfDs or RfCs.

The reality is that there is a large gap between the resources necessary to develop biologically-based models and the resources required for the less rigorous and less robust TEF, MOE, and HI approaches. To move beyond the status quo, it will be necessary to ascertain the key scientific questions and undertake appropriate research to construct and validate quantitative biologically-based models to estimate cumulative risk with an acceptable degree of accuracy (Carpenter et al. 2002, Krishnan et al. 2002). This effort will be aided by the application of new methods and technologies, such as pharmacogenomics and toxicogenomics (Carpenter et al. 2002, Thomas et al. 2002), biologically-based computer modeling (Liao et al. 2002), and improved methods for personal exposure assessment, including biological markers of exposure, effect, and susceptibility (Sexton et al. 2004, Weis et al. 2005).

Table 6-1. Comparison of Cumulative Risk Assessment Methods Applicable to Chemical Mixtures [Listed in approximate order from most preferable (1) to least preferable (6)].

Approach	Assumptions	Resource Requirements	Strengths and Weaknesses
1. Biologically Based Cumulative Risk Assessment using quantitative toxicokinetic, and toxicodynamic models	The mechanistic basis and mathematical forms of kinetic and dynamic interactions need to be known and some data need to be available to calibrate the model.	Most extensive	Can accommodate the most sophisticated mechanistic information available. However complex models can be difficult to derive, calibrate, and verify; and the extensive development process can make resulting predictions seem more robust than they really are.
2. Interactive Hazard Index using evidence and/or mathematical theory on pairwise interactions $HI_{INT}=\sum_i f(HQ)_{pair}$	Combines information on differences between simple additive, multiplicative, or other empirical functions (f) for pairs of mixture components.	Moderately Extensive – needs pairwise effect data on major mixture components.	Theoretically able to accommodate pairwise information. Specific exemplary applications of this idea have not yet been fully worked out.
3. Toxicity Equivalency Factor Approach $Dose_{TEQ} = \sum_i (dose_i\ TEF_i)$	The main assumption here is that the actions of each agent are fully represented by a single index chemical. Other effects outside of the one defined by the index chemical will not ordinarily be included.	Moderately Extensive	Analysis is simplified by treating all doses of multiple chemicals as equivalent to the weighted sum of the activity of the components of the mixture. However, the TEF derivation can present problems, e.g., TEFs for dioxin-related effects do not include a kinetic component. Expressing TEFs in 10-fold units discards relevant information..
4. Margin of Exposure Approach using TEFs $MOE = NOAEL \div dose_{TEQ}$	Assumes additivity of chemicals whose effects may in fact be either less or more than additive because of differences in modes of toxic action, nonlinearities in dose response, and differences in the timing of external exposures and internal absorption of different components of the mixture.	Moderately Extensive	Appears to avoid "extrapolations" inherent in various uncertainty factors, but there is an added responsibility to properly account for the real concerns embedded in the uncertainty factors. One-number summary of "exposure" obscures the distributional nature of exposures among people, over time and space.
5. Hazard Index Approach using NOAEL or Benchmark Dose $HI = (HQ^2)_i = \sum$ (Exposure $Metric_i/NOAEL_i$ or BMD_x)	Same as above.	Minimally Extensive	Uses measured NOAEL or BMD as a basis for comparison, but is not a true quantitative risk assessment. Single comparison value obscures scientific judgments about uncertainty factors and masks distributional nature of exposure.
6. Hazard Index Approach using Reference Dose or Concentration $HI = (HQ^2)_i = \sum$ (Exposure $Metric_i/RfD_i$ or RfC_i)	Same as above, but with the additional implicit assumption that the judgments made to translate NOAELs or BMDs into RfDs/RfCs are comparable	Minimally Extensive	Simplest approach with least resource requirements, but depends heavily on scientific judgment to translate NOAELs or LOAELs into RfD or RfCs. Not a true quantitative risk assessment, just a single

	across chemicals.		comparison value that obscures scientific judgments about uncertainty factors and masks distributional nature of exposures.

BMD_x = benchmark dose HI = hazard index HI_{INT} = interaction-based hazard index HQ = hazard quotient LOAEL = lowest observed adverse effect level MOE = margin of exposure NOAEL = no observed adverse effects levels TEQ = toxicity equivalency TEF = toxicity equivalency factor UF = uncertain factor for interactions with a default value of 10

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