

whole body/environment, compartment/whole body and compartment/threshold. If these dimensionless ratios can be correlated or predicted and their kinetics determined, this approach provides a relatively simple and quantitative expression of the chain of events resulting in toxicity.

For respiration (bioconcentration), f_B/f_E is about 1 unless dietary exposure is included (bioaccumulation), in which case f_B/f_E may exceed unity. Conversely, if rapid biotransformation occurs, then f_B/f_E may be less than 1. To a first approximation, we suspect that all the f_C values will approach f_B . As the fugacity of a chemical increases in the body, and more specifically at the target compartment, the chemical activity at the site of action increases thus increasing the likelihood of a disruptive event. Disruption occurs when f_C/f_T approaches or exceeds 1. Empirical support for these assertions is provided by whole body toxicity data for narcotic organics (de Bruijn et al. 1991; Chaisuksant et al. 1997). We have calculated the exposure and whole body fugacities as shown in Figure 1. The ratio f_E/f_B (and presumably f_E/f_T) is about 1.0. There is no such simple and direct relationship using concentrations.

The contribution of fugacity

How then can the fugacity perspective best contribute to elucidating toxicity? A threshold concentration in a compartment for a site of action where toxicity occurs can be converted to a corresponding fugacity f_T . This fugacity can be readily extended to a whole body fugacity and then to the combined exposure fugacity from an organism's food and environment. Complicating factors such as biotransformation and the kinetics of delivery, distribution and disruption must also be considered. Through the lens of fugacity, toxic effects result from prolonged exposure to external fugacities that reach the target compartment threshold fugacity.

Fugacity can help answer two related environmental questions. How best can toxicologists measure and correlate toxicity? Bioassays test various selected values of f_E until f_T is reached. If equilibrium is approached, f_E gives a direct estimate of f_T . Second, what is the risk of toxicity from a defined exposure concentration? The answer lies in the relative values of f_E and f_T . Provided f_E is much smaller than f_T there is little risk. As f_E approaches f_T the risk increases and when they are equal a toxic effect is inevitable.

So was Paracelsus wrong when he suggested that "the dose makes the poison"? This is an understandable perspective given that as a physician he was seeking to find a dose that would be beneficial therapeutically but not poisonous. The environmental situation is fundamentally different. Exposure is measured by concentrations or fugacities and times. The kinetic and dynamic processes resulting in environmental toxicity can be well quantified using fugacity. Fugacity directly expresses the relationship between chemical activities in exposure media and at the site of toxic action. Fugacity at the site of action exceeding a threshold elicits a toxic event. To the physician or pharmacist the dose does make the poison but to the environmental toxicologist it is fugacity that makes the poison. Satisfyingly, both are correct!

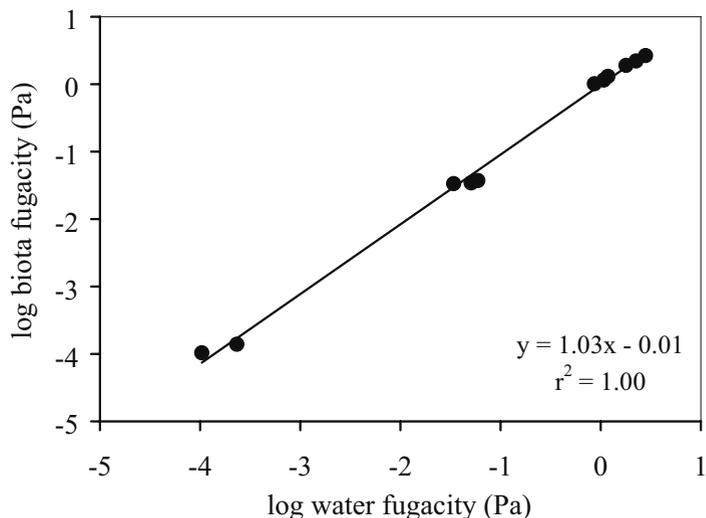


Figure 1. Quantification of acute narcotic toxicity using fugacity. Data are from de Bruijn et al. (1991) and Chaisuksant et al. (1997) for 1,2,3-trichlorobenzene, pentachlorobenzene, fenthion, and 1,4-dibromobenzene for fish of varying lipid contents.

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Decline of Sierra Nevada Amphibians: What We Know, What We Need to Know

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Amphibians are sensitive indicators of environmental health because: 1) they have moist, permeable skin that readily absorbs toxicants; 2) they spend time both on land and in the water where they are exposed to chemicals in both environments; 3) they participate in many parts of the food web from herbivorous tadpoles to insectivorous adults to prey for birds and mammals; and 4) they can constitute a significant part of the vertebrate community biomass, sometimes twice as much as the local bird

community, and equaling the small mammal community (Stebbins and Cohen, 1995). Concern about declining amphibian populations was originally voiced at the First World Congress of Herpetology where herpetologists from around the world met in 1989 and realized that many amphibian populations were being lost. This concern led to population studies in many areas around the globe. Stuart et al. (2004) recently provided a worldwide assessment of trends in amphibian populations and concluded that amphibians were declining faster than either birds or mammals. While many declines were due to habitat loss, unidentified factors threatened many of the most rapidly declining species. Field work in California has demonstrated that the Cascades frog (*Rana cascadae*), and six species of frogs and toads in the Yosemite region have all undergone significant declines (Fellers and Drost, 1993; Drost and Fellers, 1994).

While much of the initial research focused on determining whether declines were occurring, increasingly, efforts have turned to determining causes of declines. A long list of potential factors has been proposed including pesticides, disease, non-native predators/competitors, ultraviolet radiation, etc. In California, it is clear that non-native fish are impacting amphibian populations in the Sierra Nevada, but pesticides and chytridiomycosis (caused by the recently-described chytrid fungus, *Dendrobatidis batrachochytrium*) appear to be the most likely causes of broad-scale amphibian declines.

Pesticides are a concern primarily because of their use on agricultural lands, especially in the Central Valley that lies just west of the Sierra Nevada. California croplands total 40.4 million ha, 11% of the state's land area, and >7,500 metric tons of active ingredient were used on these croplands in 2003. The four most commonly used compounds were chlorpyrifos, endosulfan, malathion, and diazinon. The prevailing winds blow from the Pacific Ocean, across the Central Valley, and up into the Sierra Nevada. Pesticides applied in the Central Valley move with the prevailing winds into the adjacent mountains where they have been detected in rain, snow, air, sediment, and tissue samples from both Pacific treefrogs (*Pseudacris regilla*) and mountain yellow-legged frogs (*Rana muscosa*) (McConnell et al., 1998; Sparling et al., 2001). The pattern of amphibian decline mirrors the presence of pesticides; frogs are mostly or entirely gone from many of the drainages that face the prevailing winds, but they are still present in valleys lying perpendicular to the prevailing winds (Fellers, unpubl.).

Some California amphibians may be particularly sensitive to pesticides. In a laboratory experiment, 83% of foothills yellow-legged frogs (*R. boylei*) exposed to less than 1 ppb of endosulfan from near hatching to metamorphosis, a period of around 120 d, died and mortality was 100% at concentrations above 1 ppb. In contrast, significant mortality in Pacific treefrogs and western toads (*Bufo boreas*) was not observed below 3 ppb endosulfan. Substantially higher concentrations of chlorpyrifos were needed to produce significant mortality in *R. boylei* and *P. regilla* (Sparling, unpubl.). *In situ* experiments at Yosemite and Sequoia National Parks in California revealed higher mortality, greater incidence of deformities and significant genotoxicity compared to treefrogs raised at Lassen Volcanic National Park where the Central Valley influence is less (Cowman, unpubl.). Collectively, these data provide a substantial weight of evidence

argument that pesticides are playing a significant role in the decline of amphibian populations in California.

Amphibian declines have been ongoing for 15 to 20 years in California. We do not know of a single population that has shown a significant increase over that time, while many have declined or been extirpated. We are in an increasingly desperate need of identifying and correcting the cause of these declines.

What do we need to know to evaluate whether pesticides are contributing to amphibian declines in California, or elsewhere? First, we need to know more about the effects of commonly used pesticides on native species of frogs and toads. LC50s, LOELs and NOAELs based on environmentally realistic pesticide concentrations and exposure periods are needed to evaluate potential impacts. Endpoints including survival, time to metamorphosis, size at metamorphosis, behavior, and deformities need to be included in the experiments. *Rana muscosa* is unusual in taking up to three seasons for the tadpoles to metamorphose. This means that exposure times can range up to 30 months for the tadpole stage alone.

Amphibians in the wild are not exposed to one pesticide at a time. Amphibians in montane California are regularly exposed to combinations of the predominant pesticides. Laboratory experiments are needed to evaluate whether interactions between commonly used pesticides alter survival, compared to single pesticide exposures.

Nothing is known about the interaction between chytridiomycosis and pesticide exposure. Relyea and Mills (2001) demonstrated that the presence of a native predator increased the lethality of Carbaryl by 2 to 4 times. A similar interaction between pesticides and chytridiomycosis might well occur. These sorts of interaction experiments need to be conducted to elucidate these and other potential relationships.

Unfortunately, much of the existing data on pesticide effects are based on exposure times that are unrealistically short (e.g., 48 - 96 h), and/or involve species that are not good surrogates for amphibians in California (e.g., fathead minnows, African clawed frogs). Future research needs to use native amphibians so that results and conclusions are appropriate for wildlife that live in the areas being affected.

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