## Health Effects of Hexachlorobenzene and the TEF Approach

In her paper, van Birgelen (*1*) argued that hexachlorobenzene (HCB) should be classified as a dioxin-like compound, with a toxic equivalency factor (TEF) value of 0.0001. By doing this, HCB could add 10–60% to the total toxic equivalents (TEQs) in human milk samples in most countries. To include a compound in the TEF concept, the following criteria are used: *a*) the compound must show a structural relationship to polychlorinated dibenzo-*p*-dioxins or polychlorinated dibenzofurans; *b*) the compound must bind to the aryl hydrocarbon (Ah) receptor; *c*) the compound must elicit Ah receptor-mediated biochemical and toxic responses; and *d*) the compound must be persistent and accumulate in the food chain (*2*). van Birgelen (*1*) also referred to these criteria.

In respect to the toxic responses, one can question the validity of using a TEF approach for HCB, because the most basic assumption for this concept is that the combined effects are dose or concentration additive (*3*). The dioxin-like effects for HCB mentioned by van Birgelen (*1*) are reduction in reproduction, splenomegaly, increase in mortality, neurologic alterations, teratologic effects, and immunotoxic effects. Although TCDD and HCB share target organs of toxicity, the effects produced in these systems or organs do differ:

- Laboratory animals that are lethally exposed to TCDD die following a wasting disease that is not seen in HCB poisoning
- Neurotoxic effects such as tremors are typical after HCB exposure in rodents and birds (*4*) but are not observed after TCDD poisoning (*5*)
- Thymic atrophy and suppression of thymus-dependent immunity is a hallmark of TCDD toxicity observed in all species investigated (*6*), whereas the immunotoxicity of HCB is species-dependent: this is characterized in rats and in humans by splenomegaly, enlarged lymph nodes, and enhancement of parameters of specific immunity, and in the mouse by suppression of most immune responses (*4*)
- Target organs for the carcinogenic actions of HCB and TCDD are different; only the liver and thyroid are target organs for both compounds (*5,7*)
- Edema formation, not mentioned by van Birgelen (*1*), is a pathology typically caused by TCDD in some species, which includes subcutaneous edema, ascites, and hydropericardium in the chicken (*5*)
- Chloracne is a specifically characteristic skin disease attributed to human exposure to TCDD-like compounds, whereas the cutaneous lesions caused by HCB poisoning are hirsutism and bullous lesions, usually in sun-exposed skin areas; these skin lesions in victims of HCB poisoning have been attributed to the porphyrogenic activity of HCB (*4,7*).

Furthermore, for the toxic effects of HCB mentioned above (which differ largely from those induced by TCDD, although in the same target organs), there are no available data that show these effects to be Ah receptor mediated. These considerations do not justify the TEF approach as defined by Ahlborg et al. (*2*) for HCB.

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### References and Notes

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## Hexachlorobenzene: van Birgelen's Response

I appreciate Vos's comments about the inclusion of HCB in the TEF concept, in reference to my paper on the dioxin-like properties of HCB (*1*). Vos correctly points out that various effects of HCB are not identical to those of TCDD. However, HCB has some properties that are typical for dioxin-like compounds. These include binding to the Ah receptor and induction of cytochrome P4501A. In addition, HCB has been shown to bioaccumulate. These factors are a prerequisite for including a compound in the TEF concept, which compares the potency of a dioxin-like compound to TCDD. TEFs are consensus values based on available data on relative potency values for specific compounds (*2*). TEF values are used to estimate the total dioxin activity in environmental and human samples by multiplying the TEF value by the concentration of each compound, leading to a certain number of TEQs for each compound. The summation of all TEQs in a certain mixture expresses the total dioxin activity of the mixture. Based on the binding affinity of HCB to the Ah receptor and on *in vitro* cytochrome P4501A induction and porphyrin accumulation, I estimated a relative potency of 0.0001 for HCB (*1*).

Because HCB also has other properties, such as being a mixed-type cytochrome P450 inducer, effects that are not typical for TCDD are to be expected. Mono-*ortho*-substituted polychlorinated biphenyls (PCBs) are also mixed-type cytochrome P450 inducers; they have biologic effects that differ from those produced by TCDD, and yet they are included in the TEF concept. These PCBs induce both cytochrome P4501A and P4502B activities, which are also induced by HCB. Since mono-*ortho*-substituted PCBs are included in the TEF concept, HCB should also be included.

However, the database used to derive a TEF value for HCB is rather weak because there are currently no *in vivo* data that compare HCB to TCDD. In order to produce these data, the National Toxicology Program (NTP) has initiated subchronic studies that can be used to derive *in vivo* relative potency values. These studies will include measurement of the activities of cytochrome P4501A1 and P4501A2, and determination of tissue concentrations to estimate the total body burden. In addition, the NTP has initiated a continuous breeding reproduction study which includes end points that have been shown to be affected by TCDD and that currently drive risk assessment of dioxin-like compounds (*3*). This information should be useful in deriving a tolerable daily intake for HCB.

In conclusion, a TEF value for HCB is warranted on the basis of properties that are similar to other compounds which are included in the TEF concept. This TEF value should be based on *in vivo* studies to be most useful in the estimation of the total TEQ in human and environmental samples. By excluding HCB from the TEF concept, the total dioxin activity in human and environmental samples would be underestimated, especially in human breast milk (*1*).

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