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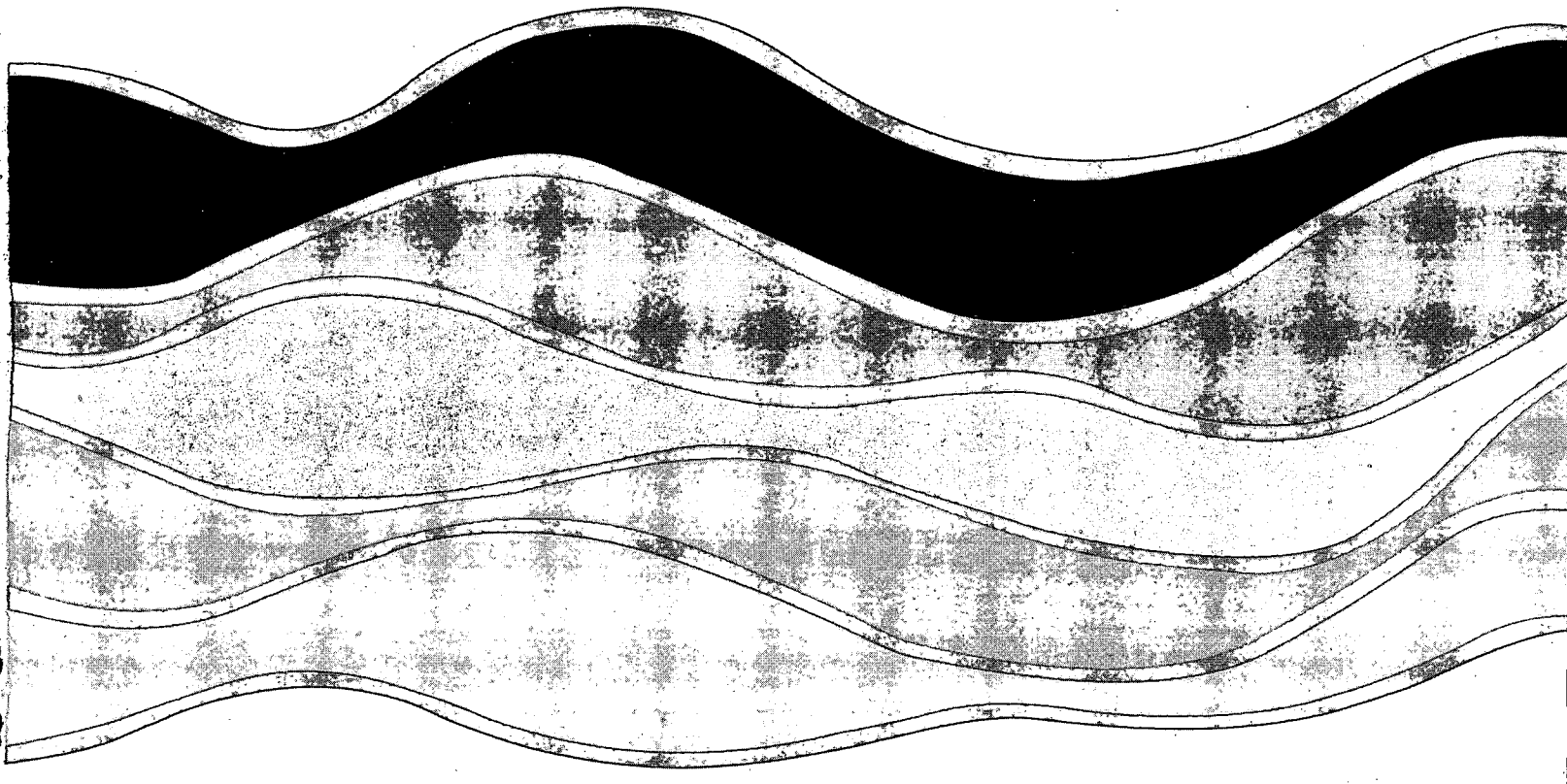

AN EVALUATION OF THE POTENTIAL USE OF MIXED FUNCTION OXYGENASE INDUCTION TO DEFINE VIRTUAL ELIMINATION OF PCDDS AND PCDFS

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An evaluation of the potential use of mixed function oxygenase induction to define virtual elimination of PCDDs and PCDFs

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Management Perspective

The definition of virtual elimination has created considerable debate. The traditional approach has been to use the chemically defined detection limits that are determined using the best available methodologies. This leads to the problem that the target for virtual elimination of a contaminant is dependent on the ever increasing improvements in analytical techniques. Although a chemical can be eliminated below some extremely sensitive chemical detection limit it might not exert biological effects at that level. The detection limits may also be much lower than background in the environment. Conversely biological responses may result from trace levels of a compound that can not be detected chemically. An alternative to the chemical endpoint may be an effects-based definition of virtual elimination. This document is a discussion of the possible application of a specific biological response (mixed function oxygenase activity) to define virtual elimination of polychlorinated dioxins and furans. In this overview, several possible approaches are explored and the advantages and limitations of each are discussed.

Sommaire à l'intention de la Direction

La définition d'élimination virtuelle a suscité un grand débat. Jusqu'ici, on a toujours utilisé les limites de détection définies chimiquement et établies à l'aide des meilleures méthodes disponibles. Cela conduit au problème suivant : la cible pour l'élimination virtuelle d'un contaminant devient tributaire des améliorations constantes dans les techniques analytiques. Bien qu'un produit chimique puisse être éliminé à une teneur inférieure à quelque limite de détection chimique extrêmement faible, il est fort possible qu'il n'exerce aucun effet nocif à cette concentration. Il est possible aussi que les limites de détection soient beaucoup plus faibles que les teneurs naturelles dans l'environnement. Inversement, des réactions biologiques peuvent être causées par des composés présents à l'état de traces, qui ne peuvent être décelées chimiquement. Une solution de remplacement au point de virage chimique : la définition de l'élimination virtuelle fondée sur les effets. Le présent document examine l'utilisation possible d'une réaction biologique spécifique (activité des oxygénases à fonction mixte) pour définir l'élimination virtuelle des dioxines et des furanes polychlorés. On donne une vue d'ensemble sur les diverses méthodes possibles, et sur les avantages et inconvénients de chacune.

Abstract

The potential use of mixed function oxygenase induction to define virtual elimination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans was examined. The basic premise was evaluated and the advantages and limitation of several approaches considered including: 1. measuring MFO in a sentinel species in the environment, 2. testing environmental extracts for MFO in cell lines, 3. using biological endpoints (MFO) to define chemical targets for virtual elimination. Although using biological endpoints is the most desirable approach to defining virtual elimination there are significant limitations to using MFO induction to define virtual elimination of PCDDs and PCDFs.

Résumé

On a examiné la possibilité d'utiliser l'induction d'oxygénases à fonction mirxte pour définir l'élimination virtuelle des dibenzo-*p*-dioxines et des dibenzofuranes polychlorés. Le principe de base a été évalué et on a analysé les avantages et les inconvénients de plusieurs méthodes, notamment : 1) la mesure d'OFM chez une espèce indicatrice dans l'environnement; 2) l'analyse des extraits environnementaux pour l'OFM dans les lignées de cellules; 3) l'utilisation de points de virage biologiques (OFM) pour définir les cibles chimiques de l'élimination virtuelle. Bien que les points de virage biologiques constituent la méthode la plus souhaitable pour définir l'élimination virtuelle, il y a des limitations significatives à l'utilisation de l'induction de l'OFM pour la définition de l'élimination virtuelle des PCDD et des PCDF.

An evaluation of the potential use of mixed function oxygenase induction to define virtual elimination of PCDDs and PCDFs

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Aim of this document

This is a document to address the question presented by the Toxics Table: "Can MFOs be used to define virtual elimination of polychlorinated dioxins (PCDD)and furans (PCDF)". We have considered the basic premise and outlined several approaches, each having both advantages and limitations. It is felt that using biological endpoints as goals for virtual elimination is ultimately the most scientifically robust way to define the levels at which chemicals have no detectable effects on the environment. However, for this specific case none of the approaches considered were fully satisfactory and all had serious limitations. Three possible approaches were identified and considered:

1. measuring MFO in a sentinel species in the environment
2. testing environmental extracts for MFO in cell lines
3. using biological endpoints (MFO) to define chemical targets for virtual elimination

Background

Although it is possible for chemicals such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) to exert negative impacts on organisms through a variety of mechanisms, Ah receptor mediated responses are the best studied and understood for this group of compounds. Binding to the Ah receptor is the first step in the process leading to a biological response to PCDDs and PCDFs. At present one of the earliest, most sensitive and consistent response to Ah receptor binding is MFO induction. MFO induction is therefore an effective and easy way to monitor binding to this receptor and can serve as an indicator of exposure to compounds which act through an Ah receptor based mechanism. It can be assumed that if MFO induction is detected then an organism is exposed to a compound that binds to the Ah receptor, and therefore could result in an Ah receptor mediated response. However, it is not known if significant biological effects occur at exposures below the threshold for MFO induction. Because the mechanisms of action of PCDDs and PCDFs is not fully understood, the question arises as to whether virtually eliminating those compounds to levels that cause only background MFO

induction is justified. The problem with using MFO as the criterion for the virtual elimination of PCDD/Fs is that it does not consider developmental effects, endocrine disruption, second generation effects, immunotoxicity, and other possible events that may occur below the threshold for MFO induction. It can therefore not be assumed that absence of MFO activity will completely ensure the absence of any effects. In addition there are compounds which can compete for the receptor and limit the expression of the expected PCDD responses.

The ultimate goal is to reduce PCDDs/PCDFs below the levels in the environment that will result in any adverse biological effects. MFO is a sensitive indicator of exposure that results in a meaningful biological event that has the potential to cause health effects. Reducing chemical burdens below the threshold for MFO induction is therefore a reasonable environmental goal. The question of whether this is low enough to protect biota from all possible effects is open to scientific debate and the question of whether the MFO threshold is too stringent and costly to achieve is a social/economic/political question. If eventually a biological effect is detected that occurs below the levels that induce MFO, this effect should become the target for virtual elimination.

There are several critical factors that must be considered if this approach is to be applied. The approach assumes that an adequate sentinel species can be selected which represents the most sensitive component of the ecosystem. The sentinel species must also represent the highest possible exposure. It is assumed that if the sentinel species is protected then the ecosystem as a whole will be also. The complexity of ecosystems makes this a difficult assumption to validate. If we wish to protect human health, wildlife, or specific components of the ecosystem, then an approach centred on a single sentinel species, such a predatory fish, may not be adequate. For example, birds or mammals feeding on fish that have PCDD/F burdens that have been reduced to a level that is considered "virtually eliminated", could show biological effects because of biomagnification. It is therefore critical that the ecosystem which is to be protected be clearly defined.

Another major consideration is the fact that MFO induction is not specific to PCDDs and PCDFs. In complex ecosystems, where mixtures of Ah-active compounds occur, MFO induction may be caused by compounds other than PCDDs and PCDFs. It is well documented that a variety of contaminants including coplanar-PCBs and PAHs, industrial effluents such as refineries, pulp mills, road runoff, etc. and natural compounds in plants can cause MFO induction. A direct cause and effect relationship between MFO induction and PCDDs and PCDFs in the environment is difficult to establish. Another complication is that in the same complex environments, mixtures of chemicals may alter the expression of MFO induction by competing for the Ah receptor or inhibiting the activity or synthesis of the protein. Exposure to PCDDs or PCDFs in complex mixtures therefore might not result in MFO induction.

Approach 1: Measuring MFO in a Sentinel Species in the environment

The first approach considered was to use MFO induction in wild animals as a defined goal for virtual elimination. MFO induction would be measured in a sentinel species which is expected to

have the highest levels of PCDDs and PCDFs, and is the most sensitive. A monitoring system in the ecosystem to be protected would include measurements of MFO induction in the sentinel species over time. Comparisons of induction to levels in the same species from pristine environments would establish the level at which virtual elimination is achieved.

Limitations:

- This approach makes the assumption that only dioxins are causing MFO induction. MFOs respond to a wide variety of compounds other than dioxins - such as chemicals in pulp mill effluents, refinery effluents, PAHs, PCBs, road runoff, etc. Thus, the MFO measured in the environment may not be linked to levels of PCDDs and PCDFs.

- Problems in defining an adequate sentinel species. Makes the assumption that a single species can be selected which will protect all components of the ecosystem.

- Problems defining the ecosystem you want to protect. Birds may have the potential to biomagnify 10 to 100 x more than fish, and if a fish species is chosen as a sentinel, we ignore this portion of the ecosystem.

- Problem defining a reference or clean sites. Need to establish a background MFO level.

- Problems with the MFO measurement itself due variation among species, season, sex, habitat and unknown biological factors.

- Problems with using MFO activity as an endpoint. The endpoint of interest is actually Ah receptor binding. MFO is a surrogate for Ah receptor binding because a sensitive, rapid and inexpensive method for measuring MFO is available.

- Compounds in complex mixtures could inhibit or compete with the Ah receptor, or could inhibit MFO activity.

Approach 2: Environmental extracts tested for MFO in cell lines

In this approach environmental samples are extracted and tested for MFO induction in a cell line. Prior to testing, extracts can be cleaned up to isolate dioxins and furans. MFO induction in cell lines exposed to extracts will be compared to MFO in cell lines exposed to extracts of reference fish.

Advantages:

- The variability in the measured endpoint in cell lines is reduced compared to wild fish. Variability in the expression of the MFO induction resulting from sex, season, and other

biological variables are controlled in the laboratory.

Limitations:

- Choice of sentinel species or environmental compartment to sample.
- Choice of reference sites or material.
- Analytical problems: how much sample do you extract? To what extent to clean-up samples?
- Which cell line to select for MFO measurement? Each cell line will differ slightly in sensitivity to different congeners.
- Improvements in the analytical techniques in the extraction and clean up, and in the sensitivity improvements to the cell line means that the target for virtual elimination is moving. As techniques get better our ability to extract and measure MFO will improve. This is a similar situation to the current chemical conundrum where improvements in methodology over the years results in an ever decreasing target for regulation of PCDD and PCDFs.
- There could still be variability in the PCDD and PCDF burdens in the environmental sentinel/compartment due to season, sex or other biological factors.

Approach 3: Using Biological Endpoints to Define Chemical Targets for Virtual Elimination

The third approach would be to use the threshold of MFO induction in sensitive species to define the target concentration of dioxins for virtual elimination. The measurement of levels of PCDDs and PCDFs in the sentinel species and the calculation of a Toxic Equivalent Quantity (TEQ) using fish-derived Toxic Equivalent Factors (TEFs), would allow calculation of a TCDD equivalent concentration in the sentinel species. This value could be compared to the threshold for MFO induction in the sensitive species (the criteria for virtual elimination). If the TEQ in the sentinel was below the threshold PCDD/F concentration for MFO induction, these compounds could be said to be "virtually eliminated" based on one of the most sensitive biological responses to dioxins and furans.

If we assume that protection of aquatic ecosystems is the goal, then we will need to protect the most sensitive species. To do this we will need to define and select the most sensitive species - and from data on effects and MFO induction by PCDD/Fs, derive a threshold concentration for which no discernible induction/effect would be expected. We will have to predict or measure PCDD/F levels in a chosen sentinel species, that will not necessarily be the same as the most sensitive species for which the threshold was derived. The criteria for deciding whether PCDD and PCDF have been "virtually" eliminated from an ecosystem will be based on the concentrations in the sentinel species (e.g., top predator, fatty fish, most likely to have high levels of PCDD/F).

For this approach there is an underlying assumption that we cannot measure PCDD/F concentrations in all species (just the selected sentinel species) and we cannot determine the threshold in all species (just what we assume is the most sensitive species). So we attempt to set the threshold for the "most sensitive" species as shown by current data (and the threshold can be modified as data on sensitivity in other species become available).

To protect an ecosystem, a suitable sentinel species is selected and PCDD/F concentrations measured in tissues. PCDD/F can be said to be "virtually eliminated" if concentrations in the sentinel species are lower than the previously defined threshold (for no MFO induction in the most sensitive species).

Advantages:

- Goal is to eliminate biological responses based on best available data.
- The chemical burden defined as virtual elimination is that which is expected to have no adverse biological effects.
- Specific to PCDDs and PCDFs.
- Multiple biological endpoints can be used to set limit (not just MFO induction).
- Considers all available information/data.

Limitations:

- Does not consider the effects of mixtures. PCDDs and PCDFs may occupy enough receptors to induce MFO to levels just below the measured threshold. Any MFO induction by PAHs or other Ah receptor-active compounds may be influenced by PCDD/F that is present at levels that would not cause MFO induction by itself. Additive effects of these Ah receptor-acting compounds may be a confounding factor. To circumvent this problem, the use of a safety factor may be appropriate - to allow setting of a threshold that takes into account the possibility (or probability) of mixtures of Ah-acting compounds in the environment.
- Scientific data are needed to select a "sensitive species" and set a threshold for MFO induction.
- Definitions are needed of a "sentinel species" appropriate for each ecosystem - and considerations of migration and exposure are necessary. We must consider which components of the ecosystem we wish to protect i.e. migratory fish, invertebrates etc. Wildlife and human health are not necessarily protected unless specifically considered.
- This approach would be difficult to apply to point sources or localized areas.

- Choice of data to calculate a lab-based threshold may not be appropriate for protecting ecosystem effects and your sentinel species.

- Extrapolation of simple, single-compound lab test data may not adequately predict effects in complex environmental situations.

- The assumption that other biological events are not occurring below the threshold for MFO may not be valid.

- A threshold for a "safe" level of PCDDs and PCDFs has not been established.

Recommendations

Although using biological endpoints is the most desirable approach to defining virtual elimination there are significant limitations to using MFO induction to define virtual elimination of PCDDs and PCDFs.

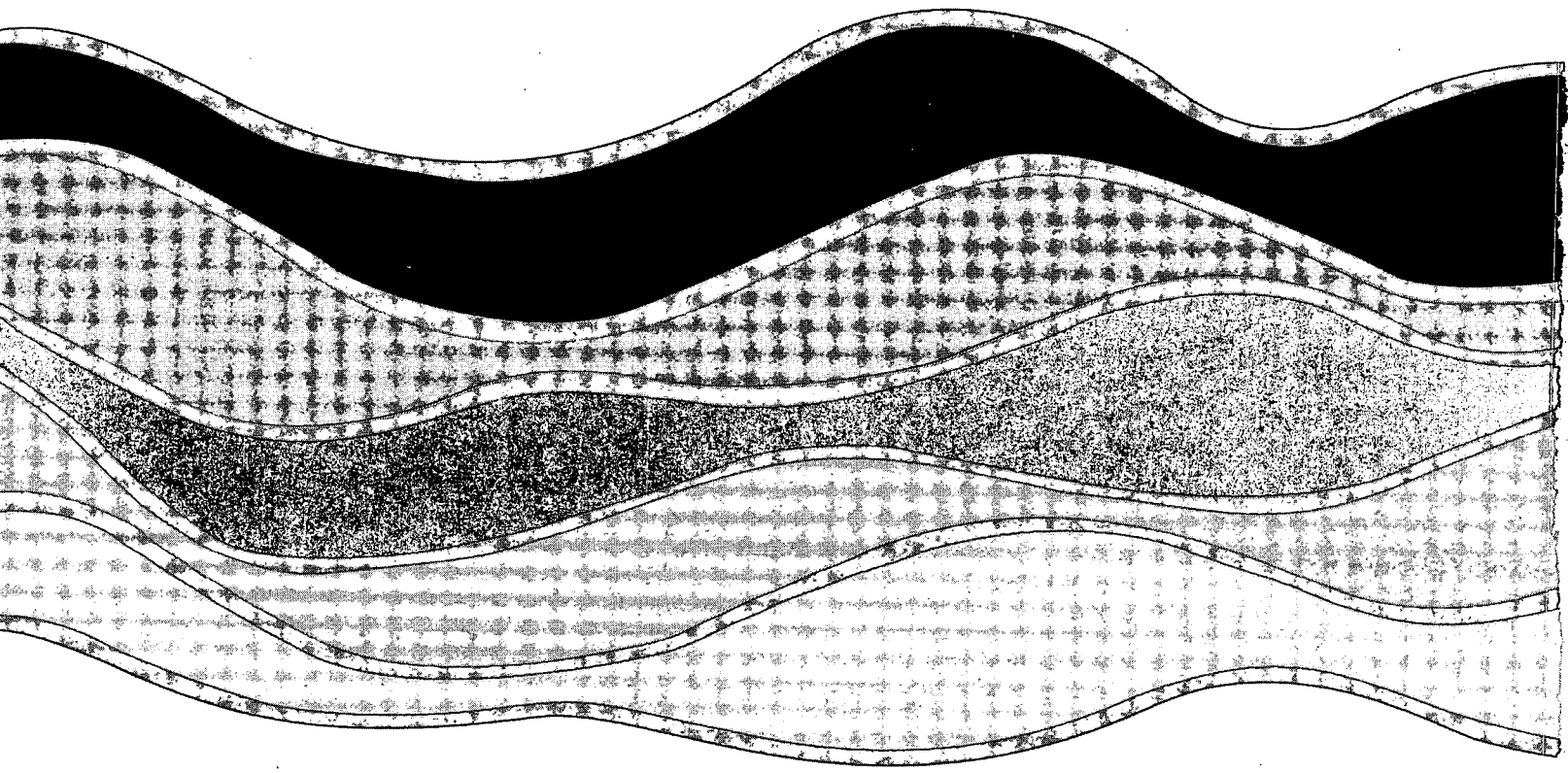
The limitations of these approaches could be reduced by research into the following issues:

- sentinel species - research into the selection of appropriate sentinel and indicator species in various ecosystems with considerations of toxicology, exposure and relevance.

- developmental toxicology - effects on developmental, immune, endocrine and behaviour in multi-generational studies.

- Mechanistic studies that would allow a better understanding of the mechanism of action of PCDD and PCDF - and to better understand effects that may not be mediated through the Ah receptor.

- Characterization of reference or "normal" levels of MFO induction.



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