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DO PCDD/PCDF STANDARD SOLUTIONS USED IN BIOASSAY- OR MASS SPECTROMETRY-BASED DIOXIN ANALYSIS POSE A RISK AS POTENTIALLY ACUTELY TOXIC TO LAB PERSONNEL?

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Introduction

Working safely in laboratories requires following a number of basic principles. In general, we have to distinguish between precautionary measures aimed to prevent possible contamination of personnel with chemicals and emergency situations, i.e., after a contamination has occurred. If technicians work alone with hazardous or inflammable chemicals, provisions must be in place that allow easy access to help in case of an incident. Particular concern has been expressed with regard to hazards associated with the handling of solutions of PCDD/Fs used for cell-based dioxin bioassays and instrumental analysis. Key phrases like the "TCDD incident in Seveso", use of "TCDD-contaminated Agent Orange in Vietnam" or the "poisoning of the former Ukrainian president Viktor Yushchenko" have been used to highlight the extreme toxicity of TCDD and to suggest that emergency phone numbers of qualified treatment clinics be available in case of a workplace incident with PCDD/F solutions. Accordingly, the question has been raised as to what levels of PCDD/Fs might cause acute effects in humans and as a result would require special precautions such as permanent supervision or the presence of a second person to help in case of an incident, in particular for analysis of low contaminated samples as feed and food.

Materials and Methods

Monitoring for the presence of PCDD/Fs and dioxin-like (dl)-PCBs in foodstuffs or feed may be performed with screening (CALUX-type cell-based bioanalytical screening methods) and confirmatory methods (GC-HRMS or GC-MS/MS). The highest calibration points are used for discussion of possible risks.

For the CALUX screening method, the highest calibration point at the EURL (3000 pmol/L culture medium) is prepared by addition of 6 µl of a solution of 100 pg TCDD/µl to 600 µl medium.

For GC-MS-based confirmatory methods, the highest calibration point at the EURL contains unlabelled PCDD/F between 0.50 pg/µl for 2,3,7,8-TCDD and 10 pg/µl for OCDD and 13C12-labelled PCDD/F between 0.50 pg/µl for 2,3,7,8-TCDD and 6 pg/µl for OCDD. 1 ml as prepared in routine contains 6 ng WHO-PCDD/F-TEQ (as sum for unlabelled and 13C12-labelled PCDD/F).

EN 16215 for determination of PCDD/F by GC/HRMS in animal feed has 10 pg/µl for unlabelled PCDD/F and mostly 5 pg/µl for 13C12-labelled PCDD/F in its highest calibration standard. 1 ml contains 48 ng WHO-PCDD/F-TEQ (as sum for unlabelled and 13C12-labelled PCDD/F).

US EPA Method 1613 was developed in 1994 for PCDD/F determination in aqueous, solid, and tissue matrices by isotope dilution HRGC-HRMS. The calibration standards CS1 – CS5 have mostly 100 pg/µl 13C12-labelled PCDD/F. The highest calibration point CS5 has mostly 1000 pg/µl for unlabelled PCDD/F (range 200 – 2000). 200 µl (as commercially available) contain nearly 520 ng WHO-PCDD/F-TEQ.

As criterion for classification of acute toxicity, LD50-data for oral or dermal intake were applied.

Results and Discussion

The assessment of possible risks is based on the evaluation of the acute toxicity of 2,3,7,8-TCDD and it is classified in hazard class category 1 (acute toxicity estimate ≤ 5 mg/kg bw). In contrast, commercially available standard solutions (with 50 µg 2,3,7,8-TCDD/ml) are in hazard class 4 regarding acute toxicity, as the solvents are considered to be the risk-defining parameter for the low concentration ranges in these mixtures.

If the definition of 'acute toxicity' as LD50 is not strictly applied, but expanded to include also chloracne as the only effect in humans established with certainty at the Seveso incident, the resulting body burden

gives an indication on the probability for this risk. For a person with 15 kg fat and a level of 10 ng TEQ/kg lipid, the hypothetical intake of 100 ng would result in a total body burden of about 17 pg TEQ /g lipid. This is about 6,000 times lower than found in the blood serum of Yushchenko or about 1,500 times lower than found in one of the two Vienna women who despite extremely high blood levels (26,000 pg/g blood fat) developed only mild facial lesions. It's about a factor of 60 below doses which would be necessary to reach levels of approximately 1,000 pg/g blood fat which according to other studies showed that chloracne may appear. The studies of individuals in Seveso indicated that the period of time for onset of chloracne in humans after TCDD exposure was between two weeks and two months. Therefore acute symptoms specifically arising as a result of PCDD/F exposure will not occur within an "8 h time window".

Furthermore, the hypothetical intake of 100 ng TCDD is based on a "triple worst-case scenario" assuming that (1) under extreme awkward conditions the whole 1 ml of the highest standard solution for the bioassay with 100 ng TCDD/ml gets onto the skin (and not onto gloves, coat, the working place or elsewhere), (2) then, as a worst case scenario, no measures are taken to remove immediately a solution from the skin, and (3) it is assumed that the 100 ng are completely absorbed into the body through the skin. Under more realistic conditions, an accidental intake would be much lower.

In comparison to the hypothetical intake of 100 ng TCDD from highest calibration point for the bioanalytical method, 1 ml of the highest calibration curve established at the EURL would result in a hypothetical intake of "only" 6 ng WHO-PCDD/F-PCB-TEQ, 1 ml of the highest calibration point for EN 16215 in 48 ng WHO-PCDD/F-PCB-TEQ and 200 µl for EPA 1613 in nearly 520 ng WHO-PCDD/F-PCB-TEQ. However, as developed in 1994, the concentration ranges of the EPA 1613 calibration standards for unlabeled and 13C12-labelled PCDD/F reflect the needs resulting from considerably less sensitive instruments at that time and from higher contamination ranges of environmental samples and are far too high for modern analysis of feed and food.

As a result, the question as to whether an acute risk exists from the PCDD/F stock solutions used in the laboratory and therefore whether it should be required that a second person is necessary for supervision or help in case of an incident with feed or food samples or PCDD/F standard solutions under daily routine conditions for bioanalytical screening methods or GC/MS-confirmatory methods can clearly be denied. Of course, other risks like breakage of glass and work with inflammable solvents or concentrated sulfuric acid pose serious possible acute dangers which need appropriate precautions, and have to be considered generally for all kinds of laboratories. For those situations in which employees work alone with hazardous substances, adequate supervision or availability of other personnel should be ensured. This can be achieved by technical means and/or the concept of "safety partnerships", to ensure that a second person is informed about a colleague working alone and is available, if necessary. Of course, following the general precautionary principle, measures should still be in place to prevent contamination of personnel from chemicals not only from the perspective of acute intoxication but also with regard to long-term effects. However, these do not require the permanent supervision of a person working alone.