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Review

Do PCDD/PCDF standard solutions used in dioxin analysis pose a risk as potentially acutely toxic to lab personnel?

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HIGHLIGHTS

- Evaluation of dioxin standards as potentially acutely toxic for lab personnel.
- General aspects of acute toxicity and related hazard categories regarding dioxins.
- Evaluation of need for a second person at spiking of dioxin standard solutions.
- Balancing of potential acute risks in a lab “dioxins vs other chemicals”.
- Support of precautionary measures to avoid long-term adverse health effects.

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ABSTRACT

Laboratory safety requires protecting personnel from chemical exposures. Working with stock solutions of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDFs) in routine analysis of feed and food with bioanalytical or physicochemical methods raises some concerns. Since PCDD/PCDFs are considered as possibly acutely toxic, the potential risks were evaluated to determine whether supervision of their use is necessary. Based on LD_{50}-data for oral or dermal intake, hazard classification of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a substance (category 1) and in commercially available TCDD standard solutions (category 4) is different. As worst case exposure scenario during routine laboratory work it was assumed that a dose of 100 ng TCDD gets onto the skin and is absorbed. This would result in the total body burden of a 70 kg person with 15 kg fat increasing from 10 (upper range of current background levels) to ~17 pg of toxic equivalents (TEQs) of PCDD/PCDFs per g lipid, a level commonly observed over past decades. Chloracne, the main acute effect occurring weeks after exposure, is observed at much higher blood concentrations than estimated from accidental laboratory exposure. Immunotoxicity, developmental effects and other toxic effects may occur at lower blood levels, but require longer periods to develop. Since acute toxic symptoms don’t occur within an “8 h acute time window”, no supervision is necessary when working with standard solutions in routine analysis. Nevertheless, precautionary measures are needed regarding long-term adverse health effects and appropriate workplace conditions must exist to ensure that additional occupational exposure to PCDD/PCDFs by laboratory personnel is negligible.

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Contents

1. Introduction

Working safely in laboratories requires individuals to follow a number of basic principles and to respect guidelines as issued e.g. by the German employer's liability insurance association for the chemical industry (Berufsgenossenschaft Rohstoffe und chemische Industrie, no date) or the American Chemical Society (American Chemical Society, 1995). 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/spill has occurred. Important precautionary measures comprise: working in a fume hood; a good ventilation system in the laboratory; protection with laboratory coat, safety glasses and gloves, especially when working with certain standard solutions; no consumption of food or drink. These measures are adequate also for analysis of hazardous chemicals in food and feed. High concentrations of hazardous chemicals, more typically found in environmental samples, in particular after accidents, might require additional precautionary measures. Also if technicians work alone with hazardous or inflammable chemicals, the opportunity to get help in case of an incident has to be planned.

Dioxin-like (dl) compounds comprise 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) and 16 other polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) with chlorine-substitution in the 2,3,7,8-position and 12 polychlorinated biphenyls (PCB). These compounds all have being assigned a Toxic Equivalency Factor (TEF) by World Health Organization (WHO) expert groups (van den Berg et al., 2006). Monitoring for the presence of PCDD/PCDFs and dl-PCBs in food or feed stuffs may be performed with both screening and confirmatory methods (Commission Regulation (EU) No 2017/644; Commission Regulation (EU) 2017/771). After extraction and clean-up, screening and confirmatory methods can be applied: CALUX-type bioanalytical screening methods determine dioxin-like compounds by a cell-based detection system, the above EU regulations for analytical criteria allow also use of GC/MS for screening. Confirmatory methods are based on GC-HRMS or GC-MS/MS. In the "Field of application" section, the goals and possible use of data (including also limitations of use) achieved by screening and confirmatory methods are defined. Thus, the potential hazards originating from chemicals (PCDD/PCDFs, organic solvents, sulfuric acid, absorbents) and cells (in case of CALUX-type screening methods) have to be considered.

When working with chemicals in a laboratory, an important question is which chemicals are acutely toxic and under what conditions. If there are acutely hazardous chemicals present, the characteristics of the work situation shall be defined with respect to the dose, i.e., concentration of the hazardous chemical under consideration, the volumes used and the exposure pathway/time and the resulting possible intake, including definition of contact (ingestion [+mass], inhalation [+volume], skin [+area]). For the assessment of the risk for the technicians, the time until an intervention to either eliminate the source of exposure or to provide medical assistance/countermeasures needs to be taken into consideration. Based on such an evaluation, the question can be answered whether a permanent supervision by a second person should be considered, which in case of an incident could help immediately, as might be necessary when working with chemicals like neurotoxicants (as an example for direct and incapacitating effects of a chemical with different toxic properties in comparison to dioxins and other halogenated aromatic hydrocarbons). In this case compounds may cause immediate intoxication which, if the relevant doses are exceeded, could even be lethal unless the affected person is immediately treated with an antidote.

Particular concern has been expressed with regard to working with PCDD/PCDF and dl-PCB solutions in a dioxin laboratory with responsibilities for food and feed control. Keywords like the TCDD

incident in Seveso, use of TCDD-contaminated agent orange in Vietnam or the poisoning of the former Ukrainian president Viktor Yushchenko were used to highlight the extreme toxicity of TCDD and to propose the availability of phone numbers for emergency calls in case of an incident in such laboratories for further treatment in qualified clinics. Later in the following discussions, aspects of development toxicity, hormonal effects, liver effects and immunotoxicity were also addressed and seen as relevant for acute toxicity. Furthermore, not only was 2,3,7,8-TCDD characterized by IARC as carcinogenic to humans (group 1), but also 2,3,4,7,8-pentachlorodibenzofuran and 3,3′,4,4′,5-pentachlorobiphenyl (IARC, 2012). Later, All PCBs (dioxin-like and non-dioxin-like) were classified into group 1 (IARC, 2016).

Certainly, these safety aspects are relevant also to other fields of dioxin analysis, e.g. for biological samples in general or for environmental samples, and thus are of interest to a larger group of scientists and technicians. This evaluation might be useful as orientation, allowing comparison of the doses of a possible contamination of technicians as a worst case scenario under routine conditions as described in this paper with the concentrations and doses used in a particular laboratory.

As starting point for clarification, it is necessary to differentiate between acute toxicity, short-term and more long term effects (as development toxicity, hormonal effects, liver effects and immunotoxicity) that could occur months if not years after exposure. The key question was what levels of PCDD/PCDF might result in acute effects that would require special working conditions, such as permanent supervision or availability of a second person to help in case of an incident in a dioxin laboratory, particularly that involving feed and food analysis. For this particular concern, acute toxicity and short-term effects are of central importance.

2. Acute toxicity – general aspects

According to the International Labour Organization (ILO), acute toxicity refers to those adverse effects occurring after oral or dermal administration of a single dose of a substance, or multiple doses given within 24 h, or an inhalation exposure of 4 h. The criteria for classification are based on lethal dose data (LD_{50} [*lethal dose*] for oral or dermal intake, LC_{50} [*lethal concentration*] for inhalation) (International Labour Organization, no date): Category 1, the highest toxicity has cut off values for (approximate) LD_{50}/LC_{50} values of 5 mg/kg bw by the oral route, 50 mg/kg bw by the dermal route, 100 ppm for gases or gaseous vapours, 0.5 mg/L for vapours, and 0.05 mg/L for dusts and mists. These toxicity values are currently used primarily by the transportation sector for classification of packing groups. Category 5 is for chemicals which are of relatively low acute toxicity but which, under certain circumstances, may pose a hazard to especially vulnerable populations. These substances are anticipated to have an oral or dermal LD_{50} value in the range 2000–5000 mg/kg bw or equivalent doses for other routes of exposure.

The International Union on Pure and Applied Chemistry (IUPAC) in its Gold Book defines acute toxicity as follows "1. Adverse effects of finite duration occurring within a short time (up to 14 d) after administration of a single dose (or exposure to a given concentration) of a test substance or after multiple doses (exposures), usually within 24 h of a starting point (which may be exposure to the toxicant, or loss of reserve capacity, or developmental change, etc.), and 2. Ability of a substance to cause adverse effects within a short time of dosing or exposure (International Union on Pure and Applied Chemistry, 2014).

The United States Labor Department, Occupational Health & Safety Administration (OSHA) has established two levels of "occupational exposure limits" (OELs): Legally binding (permissible exposure limits, PELs) and recommended limits. PELs refer to 8-h weighted and ceiling concentrations. Furthermore, short-term exposure limits (STELs, see below) are listed (United States Labor Department, Occupational Health & Safety Administration (OSHA), no date - a).

For protection of workers, the "short-term exposure limit (STEL)" is defined as the "The maximum concentration of a chemical to which workers may be exposed continuously for up to 15 min without danger to health or work efficiency and safety" (Medical Dictionary for the Health Professions and Nursing, 2012). Accordingly, STEL is considered a hygiene regulatory standard and may be used as a legal limit in the United States for exposure of an employee to a chemical substance. The Occupational Safety and Health Administration in California has set OSHA/CAL STELs (and PELs) for a number of chemicals (State of California, Division of Occupational Safety and Health, no date).

US-EPA applies "Acute Exposure Guideline Levels" (AEGLs) to be used by emergency planners and responders worldwide as guidance in dealing with rare, usually accidental, releases of chemicals into the air. AEGLS represent threshold levels for the general public and are expressed as specific concentrations of airborne chemicals at which health effects may occur. They are designed to protect the elderly and children, and other individuals who may be susceptible (United States Environmental Protection Agency, no date). AEGLs are calculated for five relatively short exposure periods – 10 min, 30 min, 1 h, 4 h, and 8 h – as differentiated from air standards based on longer or repeated exposures. AEGL "levels" are dictated by the severity of the toxic effects caused by the exposure, with Level 1 being the least and Level 3 being the most severe. All levels are expressed as parts per million or milligrams per cubic meter (ppm or mg/m^3) of a substance above which it is predicted that the general population could experience, including susceptible individuals:

- Level 1: Notable discomfort, irritation, or certain asymptomatic non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.
- Level 2: Irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.
- Level 3: Life-threatening health effects or death.

In 2001, the National Academies published procedural guidance or "Standing Operating Procedures" to make development of AEGLs systematic, consistent, documented and transparent to the public.

3. Acute toxicity hazard categories

Regulation (EC) 1272/2008 sets criteria for classification and labelling of chemicals (substances and mixtures) (European Parliament and Council, 2008). With a view to facilitating worldwide trade while protecting human health and the environment, these criteria were harmonized taking into account 40 years of experience obtained through implementation of existing Community chemicals legislation and 12 years of development within the United Nations (UN) structure, resulting in the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

The World Health Organization (WHO) also has adopted the GHS system for the classification of chemicals (e.g., pesticides in 2009) (World Health Organization, 2009).

Health hazards as defined in Regulation (EC) 1272/2008 comprise acute toxicity, skin corrosion/irritation, serious eye damage/eye irritation, respiratory or skin sensitization, germ cell mutagenicity, carcinogenicity, reproductive toxicity, specific target organ toxicity — single exposure, specific target organ toxicity — repeated exposure and aspiration hazards.

In Regulation (EC) 1272/2008, Annex I, Part 3 "Health Hazards", and in the OSHA Hazard Communication Standard, Appendix A to § 1910.1200-Health Hazard Criteria (United States Department of Labor, Occupational Health & Safety Administration (OSHA), no date-b), acute toxicity is defined specifically as "adverse effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 h, or an inhalation exposure of 4 h".

As well in Regulation (EC) 1272/2008 as in OSHA Hazard Communication Standard, the hazard class Acute Toxicity is differentiated into: (i) acute oral toxicity; (ii) acute dermal toxicity and (iii) acute inhalation toxicity. Substances can be allocated to one of four toxicity categories based on acute toxicity by the oral, dermal or inhalation route. The criteria for classification of chemicals as acutely toxic are based on lethal dose data: Acute toxicity values are expressed as (approximate) LD_{50} (oral, dermal) or LC_{50} (inhalation) values or as acute toxicity estimates (ATE). Four acute toxicity hazard categories are defined on basis of the acute toxicity estimate (ATE) for oral and dermal exposure ranging from category 1 (most toxic substances) to category 4 (least toxic substances), with the example of category 1 as follows: oral with ATE $\leq$ 5 mg/kg bw; dermal with ATE $\leq$ 50 mg/kg bw.

4. Hazard classification of 2,3,7,8-TCDD as substance and in stock solutions

Requirements for the compilation of safety data sheets are set in a regulation of the EU Commission of 2015 (Commission Regulation (EU) 2015/830). In Section 2, the hazards of a substance or a mixture and the appropriate warning information associated with those hazards for the whole product shall be described. For the commercially available PCDD/PCDF solutions with 50 μg/ml, the solvent (e.g. > 99.99% nonane, or 89.993% nonane and 10% toluene) is considered as the risk-defining parameter of such mixtures (relative to flammability and toxicity), with acute toxicity category 4 for the whole product (99.993% of a nonane/toluene mixture and 0.007% of 2,3,7,8-TCDD) (Wellington Laboratories, 2013; Cerilliant, 2015), whereas category 1 would be applicable for 2,3,7,8-TCDD as pure substance (Sigma-Aldrich, 2015a). In Section 3, substances shall be listed as chemicals in a mixture and the individual classification of each shall be provided, if the category for the acute toxicity is 1, 2 or 3 and the concentration $\geq$0.1%, or if the category is 4 and the concentration above 1%. As the maximum concentration of 2,3,7,8-TCDD in commercially available standard solutions is 50 μg/ml and thus far below 0.1%, 2,3,7,8-TCDD is usually given as an ingredient in such TCDD-standards, but the indication of the individual hazard category of TCDD is not necessary in these safety data sheets.

According to the Technical Rules for Hazardous Substances, for 2,3,7,8-TCDD the risk of cancer has to be specified for solutions containing more than 0.0000002% (Technische Regeln für Gefahrstoffe [TRGS 905], 2014).

5. Toxicological evaluation of dioxin-like compounds

5.1. Acute toxicity

A huge number of toxicological studies were used to characterize the risk for the different groups of dioxin-like compounds under various aspects and scenarios. JECFA (the Joint Expert Committee on Food Additives of WHO and FAO) differentiates between acute toxicity, short-term and long-term toxicity and carcinogenicity (Joint FAO/WHO Expert Committee on Food Additives, 2002; Canady et al., 2002). US EPA in its comprehensive dioxin reassessment differentiates between acute, subchronic and chronic toxicity (United States Environmental Protection Agency, 2004; see here Part II, Section 3). Under "acute toxicity", both JECFA and EPA summarize studies on lethal doses expressed as LD_{50} (μg/kg bw) in various animals. This definition is in line with the Regulation (EC) 1272/2008 setting criteria for classification of chemicals (substances and mixtures) and classification of chemicals as acutely toxic based on lethal dose data (*see* section 2).

These values vary widely between and among species. For example, the median lethal dose of 2,3,7,8-TCDD in guinea pigs treated orally was 0.6 μg/kg bw, while that in hamsters was >5000 μg/kg bw. Similar conclusions are also drawn in the above cited draft US EPA report (United States Environmental Protection Agency, 2004; see here Part II, Section 3).

5.2. Acute reference dose

As most compounds do, also dioxins might cause an effect after a single high dose. In humans, the most readily observed effect is the development of chloracne, however, other chlorinated compounds can produce similar effects. Other effects seem more subtle and may be difficult to distinguish from chronic effects due to the persistence of the compounds in the body. So a single high dose may cause similar effects as a repeated low dose. Furthermore it has to be considered that the development of toxic effects might be delayed after exposure to a "toxic" concentration. Thus far, no acute reference dose for TCDD has been derived.

5.3. Chloracne

Chloracne is the best recognized effect of exposure to TCDD (see reviews, e.g. Joint FAO/WHO Expert Committee on Food Additives, 2002, United States Environmental Protection Agency, 2004 – see here [Part II Section 7 Part B]). According to a review of Bertazzi et al. (1998), at the Seveso incident in 1976, where thousands of inhabitants were exposed to TCDD, chloracne was observed in nearly 200 individuals and was initially the only effect established with certainty. At this accident, according to first estimates, quantities from hundreds of grams to a few kilograms of TCDD were released, however a re-evaluation suggests it was 34 kg or higher (di Domenico et al., 1990; see also Bertazzi et al., 1998). The presence of TCDD as the main component of the toxic cloud was made public 10 days after the accident and evacuation of the most contaminated zone A started after two weeks. In a selection of 10 children with chloracne from zone A, serum levels ranging from 828 to 56,000 ppt *(lipids)* were found. Nine adults without chloracne, from the same area, had values ranging from 1770 to 10,400 pg/g *(lipids)* (*data from* Bertazzi et al., 1998*; see also* Mocarelli et al., 1999). Thus, there was a large variability in human sensitivity and response and some individuals seemed much more "resistant" to the effects of TCDD. 5 categories between 0 (no lesions) and 4 (serious stage) were developed stratifying chloracne for Seveso residents by severity (United States Environmental Protection Agency, 2004; see here Part II Section 7 Part B). It should be stressed that chloracne was mainly observed in children, possibly related to a higher exposure but potentially also to a greater sensitivity.

In 2004, the former Ukrainian president Yushchenko was diagnosed with chloracne. Analysis of his blood showed a 2,3,7,8-TCDD level of about 100,000 pg/g lipid (Brouwer et al., 2005; Sorg et al., 2009). This concentration was between 5000- to 10,000-fold higher than the levels for the sum of PCDD/Fs and dl-PCBs observed in the general population of many countries (10–20 pg TEQ/g lipid). Assuming equal distribution in the body fat, it can be estimated that Yushchenko's intake was a few mg of TCDD.

Two food poisonings incidents, called Yusho and Yucheng,

occurred in Japan in 1968 and in Taiwan in 1979, respectively. They were caused by ingestion of rice oil that was highly contaminated with PCBs. Blood samples from five Yusho patients and three Yucheng patients were collected between 1982 and 1998 and from 1989 to 1995, respectively. TEQ levels were derived on basis of the most important PCDF congeners (without determination of dioxin-like PCBs) and estimated to have decreased from 40,000 pg/g lipid in 1969 to 600 pg/g lipid in 1997. Typical Yusho symptoms were acneiform eruption, dermal pigmentation and increased eye discharge, from which individuals very gradually recovered over time. However, enzyme and/or hormone-mediated signs of high concentrations of serum triglycerides and thyroxine, immunoglobulin disorder and others were persistently maintained for 30 years (Masuda, 2001).

In 1997, two women at a textile research institute in Vienna were somehow poisoned with 2,3,7,8-TCDD. Patient 1, who had the highest TCDD level in blood ever recorded (144,000 pg/g blood fat), developed severe generalized chloracne, whereas in the second patient, despite high blood levels (26,000 pg/g fat), had only mild facial lesions (Geusau et al., 2001; Geusau and Abraham, 2005). The authors considered the mild manifestation in Patient 2 surprising in comparison to other studies which showed that chloracne may appear at dioxin blood levels of approximately 1000 pg/g blood fat (Coenraads et al., 1999). Observations following industrial and accidental exposures have suggested that acute exposures resulting in serum concentrations of about 800 pg/g of lipid might be necessary to induce clinical effects such as chloracne, although levels in the thousands of pg/g of lipid do not always produce this effect (Centers for Disease Control and Prevention, 2016). However, in general, for a person with 15 kg body fat, an intake of 15,000 ng would be necessary to achieve a level of 1000 pg/g lipids.

No reliable information is available on the time course of occurrence of chloracne in humans after TCDD exposure. In Seveso, the eruption of blackheads was observed between 2 weeks and 2 months after the reactor release, and within 6 months, 34 cases of chloracne were identified among children (United States Environmental Protection Agency, 2004; see here Part II Section 7 Part B). Most seriously effected (categories 3 and 4) were 19 children of zone A (Signorini et al., 2000).

5.4. Other effects

Many of the effects following dioxin exposure are the same irrespective of whether the intake is acute or chronic (single or repeated exposure). For effects such as enzyme induction, immunotoxicity, developmental toxicity and a number of other toxic endpoints, the EU Scientific Committee on Food (SCF) concluded that the responses are directly associated with tissue concentrations and not simply with the daily dose (Scientific Committee on Food, 2001). The key determinants in the kinetics and the half-lives of these compounds are the absorption, the amount of fat stores in the body, the sequestration by CYP1A2 in the liver, and the rate of metabolism and excretion. From a pharmacokinetic point of view, body burden estimations are therefore considered a more appropriate dose metric for interspecies comparison than the daily dose. Similarly, JECFA concluded with regard to the relationship between human intake and doses used in studies in laboratory animals that the biochemical and toxicological effects of PCDD/PCDFs and coplanar PCBs are directly related to their concentrations in tissues and not to the daily dose (Joint FAO/WHO Expert Committee on Food Additives, 2002). Toxicokinetically, estimates of body burden are also more appropriate measures of dose for interspecies comparisons than is the daily dose. The US EPA re-evaluation of 2012 applied this principle and applied physiologically based pharmacokinetic modelling, but based it on blood rather than body burden levels. Nevertheless, under normal conditions there is an equilibrium between the levels in blood and those in the fat tissues in the body. However, accidental exposure during an incident doesn't relate to an equilibrium situation, and it could temporarily result in higher levels of exposure via transiently increased blood levels.

As described by Bertazzi et al. (1998), immunologic effects of TCDD were investigated between 1976 and 1979 in 48 children from zone A of the Seveso incident and 48 non-exposed children. These results revealed higher levels of complement activity, higher values for lymphocyte responses to phytohaemagglutinin and pokeweed mitogen, and increased numbers of peripheral lymphocytes in exposed children. However, test design limitations and poor compliance of reference children made interpretation difficult.

A comprehensive research project with particular focus on alterations in the immune system of the two highly exposed Vienna women revealed no unambiguous observations which could be linked to TCDD exposure. These results provide further evidence that immune parameters cannot be seen as sensitive biomarkers for a TCDD exposure (Abraham, 2002).

5.5. Tolerable intakes

On a global level, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is an international scientific expert committee that is administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and WHO. It provides scientific advice to the Codex Alimentarius Commission and was established in 1963 to develop harmonized international food standards and guideline codes of practice to protect the health of the consumers and ensure fair practices in the food trade. Therefore, JECFA evaluations are of particular importance at the global level. In 2001, JECFA derived a provisional tolerable monthly intake (PTMI) of 70 pg/kg bw (Joint FAO/WHO Expert Committee on Food Additives, 2002). In the EU, the evaluation of the EU Scientific Committee on Food (SCF) in 2001 is valid which derived a tolerable weekly intake of 14 pg WHO-PCDD/PCDF/PCB-TEQ/kg bw (Scientific Committee on Food, 2001).

Both tolerable intakes are comparable and are based on the accumulation of these compounds and resulting body burden. The reason to express the tolerable intake on a weekly or even monthly basis is that a relatively low daily ingestion has a small or even negligible effect on the body burden. Thus, in order to assess long- or short-term risks to health due to regular exposure to relatively low levels of these persistent substances, total or average intake should be assessed over the long-term. In both assessments, effects of TCDD on the male offspring of rats, in particular a reduced sperm production was used as the most critical effect. As such, the limits are particularly focused on preventing too high body burdens in women of child-bearing age.

In the US, the US Environmental Protection Agency (EPA) reanalyzed key issues related to dioxin toxicity and in 2012 derived an oral reference dose (RfD) of 0.7 pg per kg bw per day for TCDD (United States Environmental Protection Agency, 2012). For this analysis, the US EPA selected human data from the Seveso studies that became available in 2008. Critical effects were a reduced sperm production in men exposed as young boys during the incident, and increased levels of thyroid stimulating hormone in newborns, i.e. exposed via the mother.

SCF and JECFA applied a body burden one-compartment kinetics approach to derive an health-based guidance value (HBGV) from rat data, whereas US EPA applied physiologically based pharmacokinetic modelling of human blood levels estimated from epidemiology studies. An uncertainty factor of 3 was applied by SCF and

JECFA as the lowest-observed-adverse-effect level (LOAEL) and was close to the no-observed-adverse-effect level (NOAEL) (observed in another animal study). US EPA applied their default uncertainty factor of 10 for extrapolation from a LOAEL in the absence of a NOAEL. All three applied an additional uncertainty factor of about 3 for potential differences between humans.

6. Evaluation of risks during analysis of dioxins in feed and food

6.1. Precautionary methods for employees working alone with hazardous substances

In Germany, the regulation on hazardous substances demands a risk assessment for working with dangerous chemicals and the determination of safety precautions (Gefahrstoffverordnung, 2015). One provision requests that, if employees work alone with hazardous substances, the employer has to ensure additional precautionary methods or an adequate supervision. This can also be ensured by technical means.

At the CVUA Freiburg, in addition to technical means, the concept of “safety partnerships” was developed which should ensure that a second person is informed that a colleague is working alone and that they should be available, if necessary.

6.2. Particular precautions for working with TCDD standard solutions: suitable gloves and preparation to wipe-off drops from skin

When working with TCDD standard solutions, some of the solution can accidentally get on gloves. Thus it is important to use an appropriate type of glove, as the wrong glove can lead to chemical exposure.

For bioanalytical screening methods, TCDD standards are dissolved in DMSO. One important aspect is related to the ability of DMSO to readily penetrate gloves or skin. Therefore, the first question is the possible extent of the real contamination of a technician when some of the standard solution accidentally gets onto gloves or skin. It is suggested to use nitrile gloves, as DMSO does not penetrate them within an acceptable time period, thus protecting the individual against exposure via what would be the primary route using these solutions. Glove specifications with chemical breakthrough times for various solvents provide critical information for their suitability for use with certain solvents and purposes (Starlab, no date; VWR, no date).

The penetration through gloves or skin and intake into the body is not a matter of seconds. If some of the solution accidentally gets onto gloves, these drops can be immediately wiped off with suitable absorbing tissues and the gloves be removed and discarded without risk of a contamination of the skin protected by the gloves.

In contrast, if skin is accidentally contaminated, wiping with suitable absorbing tissues can be used in combination with tissues containing suitable solvents:

- given the chemical properties of DMSO, 1% triton x-100 is recommended followed by washing with soap and water;
- acetone or ethyl acetate would extract contaminants which are dissolved e.g. in nonane or toluene. By repeated careful cleaning of the skin with organic solvents, nearly all of the contamination can be removed from the skin before significant amounts really enter the body (it should be noted that these solvents will also extract lipids from the skin).

Nonane or toluene are the solvents for the PCDD/PCDF standard solutions. In case of an incident, few drops might get onto the skin. However, with regard to toxicological considerations, these solvents are not recommended for removal of PCDD/PCDF: Acetone or ethyl acetate are proposed due to their relatively low toxicity.

According to the EU REACH legislation, a derived no-effect level (DNEL) has to be calculated for certain chemicals as the level of exposure to a substance above which humans should not be exposed. The European Chemicals Agency (ECHA) provides data for DNEL and acute toxicity (ECHA, no date). For protection of workers in industry via the inhalation route, the DNEL for acetone is 2420 mg/m^3 for acute/short-term exposure and 1210 mg/m^3 for long-term systemic effects. With regard to the dermal exposure of workers, the DNEL is 186 mg/kg body weight/day for long-term systemic effects. Subsequently, for a person of 70 kg, the DNEL for dermal exposure would be about 13 g acetone per day. Furthermore, acetone is not classified as acutely toxic: The LD_{50} for oral intake is 5800 mg/kg in rats, 3000 mg/kg in mice, 5340 mg/kg in rabbits; LD_{50} for dermal intake is 7426 mg/kg in guinea pigs (DNEL and LD_{50} data from ECHA, 2017a, and safety data sheets of Merck, 2017a, Roth, 2016, ScienceLab.com, 2013a, and Sigma-Aldrich, 2015b).

Similarily, for ethyl acetate, the DNEL is 1468 mg/m^3 for the inhalation route for acute-systemic and acute-local effects and 734 mg/m^3 for chronic-systemic effects, and 63 mg/kg for dermal exposure and chronic-systemic effects. Furthermore, ethyl acetate is not classified as acutely toxic: The LD_{50} for oral intake is 5620 mg/kg in rats, 4100 mg/kg in mice and 4935 mg/kg in rabbits; the LD_{50} for dermal intake is > 18000 mg/kg in rabbits (DNEL and LD_{50} data from ECHA, 2017b, and safety data sheets of Merck, 2017b; Roth, 2015; ScienceLab.com, 2013b, and Sigma-Aldrich, 2015c).

The use of these solvents on suitable tissues in order to wipe off some drops of a PCDD/PCDF containing solution might result in an intake of some quantity (mg) of these solvents. Of course, it is the goal to avoid any unnecessary intake of solvents. However, this intake would be far below of levels of concern. The advantage of quick and efficient removal of accidental contamination of skin by PCDD/PCDFs significantly outweighs possible disadvantages of using these solvents. The example of use of acetone or ethyl acetate in cosmetics applied to human skin (e.g. nail polish and nail polish remover) might illustrate the low toxicological risk.

Therefore, consideration of such tools (in addition to the general measures of precaution like working in a hood with windows closed, wearing a coat and gloves) are important immediate measures that can be used in case of an accidental contamination of gloves or skin.

6.3. Possible risks from food or feed samples

In the field of analysis for determination of dioxin levels in feed and food, usually samples are contaminated with chemicals in ranges which are typically orally consumed by humans and animals. Consumption of food or feed with such low levels of contaminants does not cause acute or chronic toxic effects. Thus, also amounts of dioxins extracted from such food or feed samples would certainly not cause acute toxic effects. This even applies to highly contaminated samples obtained from an incident or e.g. certain clay materials. However, increased awareness and special measures may be taken in such cases, which are also required to avoid cross-contamination of other samples.

6.4. Possible risks from standard solutions for bioanalytical screening methods

For determination by bioanalytical screening methods, normally standard curves with TCDD are prepared. This includes relatively high levels to allow curve-fitting, aiming at a full dose-response

curve. As alternative approach, calibration with reference samples is possible. An important question is whether work with standard solutions for standard curves under routine conditions can cause acute toxicity that requires the immediate availability of a second person to be able to help in case of an accident.

The example of the following highest calibration point in a bioanalytical standard curve as used by the EU Reference Laboratory can be selected for discussion of possible risks: preparation of the "3000 pmol/L culture medium" control sample by addition of 6 µl of a solution of 100 pg TCDD/µl to 600 µl culture medium (600 pg/600 µl = 1 pg/µl = 1,000,000 pg/L = 3125 pmol/L [molecular weight of 320]). With this stock concentration, two exposure scenarios can be evaluated.

6.4.1. Contamination of skin with spiked amount of highest standard solution

If the 6 µl of the highest standard solution which are used for preparation of the highest calibration point were to accidentally get onto the skin and are not removed by the wiping off as described above, and assuming 100% absorption, a worst case intake of 600 pg TCDD has to be calculated. This 600 pg intake for a 70 kg person would result in an intake of 8.6 pg TCDD/kg bw. This is about 60% of the tolerable weekly intake derived by EU SCF and about 12% of the provisional tolerable monthly intake derived by JECFA (both of which are derived for long-term intake). No acute or even chronic toxicity would be expected to result from such a dose level.

6.4.2. Contamination of skin with whole 1 ml of highest standard solution

As "triple worst-case scenario" under routine conditions, it might be assumed that:

1) Under extreme awkward conditions the whole 1 ml of the highest standard solution with 100 ng TCDD/ml gets onto the skin (and not onto gloves, coat or the other work surfaces),
2) Additionally, in the worst case scenario, no measures are taken to immediately remove the solution from the skin, and
3) Finally all 100 ng are completely absorbed into the body.

An uptake of 100 ng TCDD into a 70 kg person corresponds to 1.43 ng/kg bw or 1430 pg/kg bw. This is about 100 times the derived tolerable weekly intake or 20 times the tolerable monthly intake.

As there is currently no acute reference dose, a comparison with the existing body burden has to be used. For a person with 15 kg fat and a level of 10–20 ng TEQ/kg lipid, the intake of 100 ng TCDD would be lower than the existing body burden of 150–300 ng TEQ. The total body burden would be increased to about 17–27 pg TEQ/g lipid, a background level that has been observed in many people over the past several decades.

In comparison with doses resulting in chloracne, this resulting total body is about 4000 to 6000 times lower than that found in the blood serum of Yushchenko (100,000 pg/g fat) or about 1000 to 1500 times lower than found in one of the two Vienna women which developed mild facial lesions (26,000 pg/g fat). It's also between 70- and 600-fold lower than that found in 9 adults from zone A in Seveso that did not have chloracne. The lower factor of 50 for children in Seveso is in line with the factor of about 60 which was derived from comparison to other studies that showed that chloracne may appear at dioxin blood levels of approximately 1000 pg/g blood fat or higher.

As a result, even under these hypothetical "triple worst case conditions" personal contamination with 100 ng TCDD is orders of magnitude lower than levels known to cause chloracne, the only clear "short-term" effect in humans.

6.5. Possible risks from calibration curves for GC/MS-based confirmatory methods

6.5.1. Calibration curves established at the EU Reference Laboratory

The EU Reference Laboratory has established a five-point calibration curve with native PCDD/PCDFs with the highest calibration point in the range between 0.50 pg/µl for 2,3,7,8-TCDD and 10 pg/µl for OCDD and $^{13}C_{12}$-labelled PCDD/PCDFs in the range between 0.50 pg/µl for 2,3,7,8-TCDD and 6 pg/µl for OCDD. One µl of this solution contains 6 pg WHO-PCDD/PCDF-TEQ, and 1 ml as prepared in routine methods contains 6 ng WHO-PCDD/PCDF-TEQ (as the sum for the native and $^{13}C_{12}$-labelled PCDD/PCDFs).

Even if the "triple worst case-scenario" is assumed as described for use of the highest calibration concentration from bioanalytical screening methods (see 6.4.2) and if the whole 1 ml of the highest calibration point of the five-point calibration curve gets onto the skin, is not removed and after some time completely absorbed, "only" 6 ng TEQ would be absorbed instead of the 100 ng with the highest CALUX standard concentration. Thus, the resulting intake would be about 4% of the existing body burden and around 6 orders of magnitude less than the dose which contaminated Yushchenko.

6.5.2. Calibration curves for standard method EN 16215

The EU-RL/NRL network contributed to the development of a method for determination of dioxins and PCBs by GC/HRMS in animal feeding stuffs published as European Standard (European Committee for Standardization, 2012). The highest calibration standard has 10 pg/µl for the native PCDD/PCDFs and normally 5 pg/µl for $^{13}C_{12}$-labelled PCDD/PCDFs. Thus, 1 µl of this solution contains 48 pg WHO-PCDD/PCDF-TEQ, and 1 ml contains 48 ng WHO-PCDD/PCDF-TEQ (as sum for the native and $^{13}C_{12}$-labelled PCDD/PCDFs).

Even if the hypothetical "triple worst case-scenario" is assumed and if the whole 1 ml gets onto the skin, is not removed and is completely absorbed, the dose would still be about half of that of the highest CALUX method standard, resulting in a total body burden of about 13 pg TEQ/g lipid (increase of about 30%).

6.5.3. Calibration curves for US EPA method 1613

US Environmental Protection Agency (US EPA) Method 1613 gives valuable recommendations for use of native and $^{13}C_{12}$-labelled PCDD/PCDFs and for the chromatographic and MS parameters for determination of PCDD/PCDFs by HRGC/HRMS (United States Environmental Protection Agency, 1994). However, as developed in 1994, the concentration ranges of the calibration standards reflect the needs resulting from considerably less sensitive instruments at that time and from higher contamination ranges of environmental samples. The calibration standards CS1 – CS5 normally have 100 pg/µl $^{13}C_{12}$-labelled PCDD/PCDFs which is far too high for modern analysis of feed and food. The highest calibration point CS5 has normally 1000 pg/µl for native PCDD/PCDFs (range 200–2000). Thus, 1 µl of this solution contains 2597 pg WHO-PCDD/PCDF-TEQ, 200 µl (as commercially available) contains nearly 520 ng WHO-PCDD/PCDF-TEQ.

If the same "triple worst case-scenario" is assumed as described above, and if the whole 200 µl would get onto the skin, is not removed and is completely absorbed, then the dose would be about five times that of the highest concentration CALUX method standard, resulting in a total body burden of about 45 pg TEQ/g lipid (an increase of about 350%).

7. Avenues to further reduce possible risks

In addition to the usual precautions (see section 6.1) and measures to remove TCDD and other PCDD/PCDFs after accidental

contamination of the skin, and the use of solvent resistant gloves (see section 6.2), the risk could be further reduced:

- For bioanalytical screening procedures: alternative approach (use of reference samples with chemical concentrations around the maximum and action levels); reduction of the volume of the standard solutions (e.g. from 1 ml to smaller volumes) used for preparation of the highest calibration standards;
- For GC/MS-based confirmatory methods: calibration curves with concentration ranges as established at the EU-RL or recommended by EN 16025 – avoidance of far too high concentration ranges of $^{13}C_{12}$-labelled PCDD/PCDF as internal standards for feed and food and of these $^{13}C_{12}$-labelled PCDD/PCDF levels and levels of native PCDD/PCDF in calibration standards.

These considerations might help to not only reduce any possible risks as preventive measures from a toxicological point of view for protection of the personnel working in a dioxin laboratory, but also from environmental aspects, including disposal of contaminated waste.

8. Conclusions with regard to use of PCDD/PCDF standard solutions and the risk of potential acute toxicity to lab personnel

All precautionary measures are supported to avoid a possible contamination of personnel working in laboratories for dioxin analysis, not only for possible acute toxic effects, but also for possible long-term adverse effects. It is highly recommended to observe general precautionary and safety measures, including working in a fume hood with closed windows when using opened standard solutions, wearing laboratory coats, safety glasses and suitable gloves and to be prepared to immediately wipe off any drops which accidentally get onto gloves or the skin. The penetration through gloves or skin and absorption into the body does not occur in a matter of seconds. If parts of the solution get accidentally on gloves, these drops can be immediately wiped off with suitable absorbing tissues and the gloves be taken off without risk of exposure or further contamination. Skin contamination should be reduced immediately and significantly by wiping off with suitable absorbing tissues in combination with tissues containing suitable solvents (in case of DMSO-solved contaminants 1% triton x-100, followed by washing with soap and water, or in case of solutions in nonane or toluene e.g. with acetone or ethyl acetate). Furthermore, the amount of standard solutions in the daily routine should be limited to a necessary minimum.

For risk analysis, an obviously unrealistic “triple worst case scenario” situation for skilled technicians in a dioxin laboratory for food and feed analysis was assumed, including: (i) that under extreme awkward conditions the whole one ml of the highest concentration standard solution of 100 ng TCDD/ml gets onto the skin of a technician (and not onto e.g. gloves, coat or work surfaces), (ii) no measures are taken to remove the solution from the skin, and (iii) that the 100 ng are completely absorbed by the body. Such an intake would result from the highest calibration standard concentrations for the standard curve for CALUX screening methods as established at the EU Reference Laboratory. In comparison, the intake for GC-HRMS-based confirmatory methods varies between “only” 6 ng TEQ from one ml of the highest calibration point of EU Reference Laboratory, 48 ng TEQ from the EN 16215 method for animal feeding stuffs and 520 ng TEQ from US EPA Method 1613, the latter of which was developed in 1994 at times of considerably less sensitive instruments and the need for higher standard concentrations because of higher contamination ranges of environmental samples.

Criteria for classification of acute toxicity are based on LD_{50}-data for oral or dermal intake. For 2,3,7,8-TCDD, these vary widely between and among species, e.g. 0.6 μg/kg bw for guinea pigs and >5000 μg/kg bw for hamsters. As a result of the evaluation of its acute toxicity, 2,3,7,8-TCDD (as a “pure” substance) is classified in hazard class category 1 (acute toxicity estimate $\leq$ 5 mg/kg bw). In contrast, the 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 (with regard to both flammability and acute toxicity) for the low concentration ranges in these mixtures.

If the above mentioned definition of ‘acute toxicity’ as LD_{50} is not strictly applied, but also expanded to include chloracne as only effect established with certainty at the Seveso incident in 1976 after massive exposure to 2,3,7,8-TCDD, different aspects have to be evaluated. For a 70 kg person with 15 kg fat and 10 pg TEQ/g lipid as the upper range of current background levels in many countries, the hypothetical intake of 100 ng would result in an increase of the total body burden to about 17 pg TEQ/g lipid. This concentration is about 6000 times lower than that found in the blood serum of Yuchenko and about 1500 times lower than that found in one of the two Vienna women which despite heavy intoxication (26,000 pg/g blood fat) developed only mild facial lesions. This level is also about a factor of 60 below those doses which would be necessary to result in levels of approximately 1000 pg/g blood fat, a concentration that may result in chloracne.

For other effects such as enzyme induction, immunotoxicity, development effects and a number of other toxic endpoints, the responses are directly associated with tissue or blood concentrations and not with the daily dose.

For development of chloracne in the context of safety in a dioxin laboratory, in particular with regard to the question of the necessity of having permanent supervision or the availability of a second person to help in case of an incident, both the doses of TCDD and the time course of toxicity in humans following TCDD exposure are important. Results from the Seveso incident suggest chloracne developed between two weeks and two months after exposure. Even under the assumed “triple worst case” scenario (with an uptake of 100 ng which results in blood levels that are 60-fold lower than seen necessary for development of chloracne), it is unlikely that any acute symptoms would occur within a “8 h acute time window” following exposure, which makes the necessity of a second person questionable.

As a result, the decisive questions were whether there is a particular dioxin-related acute toxicity risk with handling of samples or standard solutions under daily routine conditions for bioanalytical screening methods or GC/MS-confirmatory methods for PCDD/PCDF contamination in food and feed and whether a second person is necessary for supervision or to help in case of an exposure. The clear answer to these questions is “no”. However, other safety risks like breakage of glass and work with flammable solvents or concentrated sulfuric acid are serious possible acute dangers which do need appropriate precautions, and have to be considered generally for laboratories. If the safety concern for working with PCDD/PCDF solutions is transferred to other chemicals and the “triple worst case” scenario is assumed for handling of these materials (e.g. 100 ml of toluene, dichloromethane or concentrated sulfuric acid), then an exposed individual would have an immediate and severe acutely toxic problem. Thus, working in a dioxin laboratory for feed and food analysis does not pose a specific acute dioxin-related risk. For laboratories in other fields of dioxin analysis, e.g. for biological or environmental samples, this evaluation might be useful as orientation, allowing comparison of the doses of a possible contamination scenario under their specific conditions using these examples for food and feed.

For those situations in which employees work alone with hazardous substances, adequate supervision or the 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 that they are available, if necessary.

9. Considerations with regard to possible long-term low dose occupational exposure of lab personnel

The starting point for this evaluation was the question of the possibility of dioxin intake as result of an accidental exposure in a "triple worst-case scenario" and the risk of potential acute toxicity to lab personnel. In addition, it is important to establish precautionary measures, as well, avoiding low-dose occupational exposure throughout the entire professional career of an individual as much as possible.

Generally, humans are exposed to dioxins and/or PCBs through either (i) accidental exposure, (ii) occupational exposure, or (iii) environmental exposure. Several routes are possible for environmental (background) exposure, including: (i) food consumption, (ii) inhalation of air and ingestion of particles from air, (iii) ingestion of contaminated soil, and/or (iv) dermal absorption. While the last three routes normally contribute less than 10% of the total daily intake, more than 90% of human dioxin exposure derives from food (EU Commission, Scientific Committee on Food, 2000).

Thus, an important pillar in reducing exposure is the prevention of any oral intake of PCDD/PCDF. In practice, if no food is stored or consumed in a laboratory, the risk of an accidental contamination of food for consumption by a technician can be excluded.

Additionally, the risk of inhalation is quite low if accidental spills of PCDD/PCDF-containing solutions onto surfaces in the laboratory are wiped off, as described for cleaning of the skin in section 6.2 and if the general measures of precaution are observed (in particular, sufficient venting in the lab and working in a hood): At room temperature, PCDD/PCDF are not sufficiently volatile, and a sufficiently high exchange rate of air (blowing in fresh air; removing exhausted air e.g. via hoods where accidental spills might happen) allows work to occur in a good and safe lab environment. General measures avoiding elevated levels of organic solvents in the air of a laboratory (as otherwise a possible critical factor in residue laboratories) will also minimize intake of the much less volatile PCDD/PCDF.

The highest risk comes from an accidental spill of PCDD/PCDF-containing solutions (either from standards or extracts) onto the skin of personnel. Therefore, the measures of precaution as described generally in the introduction and more specifically in section 6.2 are of extreme importance in order to avoid such an accidental intake. Furthermore, the starting point for this evaluation was the concern expressed by the unlikely accidental exposure to the full amount of the highest calibration solution in feed and food analysis, with no effort to reduce this contamination from the skin. It is much more likely that accidental exposure to some drops of lower concentration ranges of PCDD/PCDFs could get onto the skin which then would be wiped off quickly and efficiently. However, since it is not possible to estimate the probability and frequency of such incidents, their details (how much was spilled, how efficiently reduced) and their consequences (resulting intake) throughout an entire professional career of an individual in a dioxin laboratory, the potential for chronic toxicity cannot be assessed. Food and feed samples are usually contaminated with PCDD/PCDF levels in ranges which are consumed by humans or animals. In particular work with highly contaminated environmental samples or high concentrations of PCDD/PCDF standard solutions requires much more care. In case of an occupational intake, the increase in the total body burden is a more useful tool as first step for estimation of possible toxicological effects.

As a conclusion, precautionary measures are needed in order to avoid occupational exposure of laboratory personnel to chemicals throughout their entire professional career. In dioxin laboratories, appropriate workplace conditions are important to ensure that any exposure of personnel to PCDD/PCDFs is negligible relative to background exposure levels. This is a critical issue, not only from the acute toxic perspective, but also with regard to possible long-term adverse health effects of these compounds.

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References

Abraham, K., 2002. Exposition gegenüber Dioxinen und verwandten Substanzen – ein Risiko für Säuglinge? Habilitationsschrift, Charité Medizinische Fakultät der Humboldt-Universität Berlin.

American Chemical Society (ACS), 1995. Guide for Chemical Spill Response Planning in Laboratories. http://www.acs.org/content/acs/en/about/governance/committees/chemicalsafety/publications/guide-for-chemical-spill-response.html.

Bertazzi, P.A., Bernucci, I., Brambilla, G., Consonni, D., Pesatori, A.C., 1998. The Seveso studies on early and long-term effects of dioxin exposure: a review. Environ. Health Perspect. 106, 625–633.

Berufsgenossenschaft Rohstoffe und chemische Industrie (BG RCI), no date. Working Safely in Laboratories. Available in German at http://www.laborrichtlinien.de and in English at http://www.guidelinesforlaboratories.de.

Brouwer, A., Botschuiver, S., Veerhoek, D., Besselink, H.A., Hamm, S., Traag, W., Zimpfer, M., 2005. Observation of an extremely high dioxin level in a human serum sample from Ukraine by DR CALUX® which was confirmed to be 2,3,7,8-Tetrachlorodibenzo-p-dioxin by GC-HRMS. Organohalog. Compd. 1705–1708.

Canady, R., Crump, K., Feeley, M., Freijer, J., Kogevinas, M., Malisch, R., Verger, P., Wilson, J., Zeilmaker, M., 2002. Polychlorinated Dibenzodioxins, Polychlorinated Dibenzofurans and Coplanar Polychlorinated Biphenyls, in WHO Food Additives Series 48 "Safety Evaluation of Certain Food Additives and Contaminants". prepared by the Fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organization, Geneva - IPCS-International Programme on Chemical Safety, pp. 454–664.

Centers for Disease Control and Prevention (CDC), 2016. National Biomonitoring Program, Biomonitoring Summary. https://www.cdc.gov/biomonitoring/DioxinLikeChemicals_BiomonitoringSummary.html (Last update: 23 December 2016).

Cerilliant, 2015. Analytical Reference Standards, 2,3,7,8-TCDD (Product Number ED-901), Safety Data Sheet, Version 5.0 revision date 06/11/2015.

Coenraads, P.J., Olie, K., Tang, N.J., 1999. Blood lipid concentration of dioxins and dibenzofurans causing chloracne. Br. J. Dermatol. 141, 694–697.

Commission Regulation (EU) 2015/830 of 28 May 2015 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Official Journal of the European Union, L 132/8.

Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2015, Official Journal of the European Union, L 92/9.

Commission Regulation (EU) 2017/771 of 3 May 2017 amending Regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls, Official Journal of the European Union, L 115/22.

di Domenico, A., Cerlesi, S., Ratti, S., 1990. A two-exponential mode to describe the vanishing trend of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in the soil at Seveso, Northern Italy. Chemosphere 20, 1559.

ECHA (The European Chemicals Agency), no date, 2017a. Acetone. https://echa.europa.eu/ECHA. https://echa.europa.eu/brief-profile/-/briefprofile/100.000.602. https://echa.europa.eu/registration-dossier/-/registered-dossier/15460/7/1.

ECHA, 2017b. Ethyl acetate. https://echa.europa.eu/brief-profile/-/briefprofile/100.005.001. https://echa.europa.eu/registration-dossier/-/registered-dossier/15437/7/1.

European Committee for Standardization, 2012. European Standard EN 16215, Animal Feeding Stuffs –Determination of Dioxins and Dioxin-like PCBs by GC/HRMS and of Indicator PCBs by GC/HRMS.

European Parliament and Council, 2008. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures. Off. J. Eur. Union L 353/1, 31.12.2008.

EU Commission, Scientific Committee on Food, 2000. Opinion of the SCF on the Risk

Assessment of Dioxins and Dioxin-like PCBs in Food, Adopted on 22 November 2000. SCF/CS/CNTM/Dioxin/8 Final, 23 November 2000. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out78_en.pdf.
Gefahrstoffverordnung, 2015. Gefahrstoffverordnung vom 26. November 2010 (BGBl. I S. 1643, 1644), zuletzt geändert durch Artikel 2 der Verordnung vom 3. Februar 2015 (BGBl. I S. 49).
Geusau, A., Abraham, K., Geissler, K., Sator, M.O., Stingl, S., Tschachle, E., 2001. Severe 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) intoxication: clinical and laboratory effects. Environ. Health Perspect. 109, 865–869.
Geusau, A., Abraham, K., 2005. Severe 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) intoxication: a follow-up of the patients from Vienna. Organohalog. Compd. 67, 1702–1704.
IARC, 2012. 2,3,7,8-Tetrachlorodibenzo-para-dioxin, 2,3,4,7,8-pentachlorodibenzofuran, and 3,3' ,4,4',5-pentachlorobiphenyl. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Chemical Agents and Related Occupations, vol. 100F, pp. 339–378. Lyon, France. http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-27.pdf.
IARC, 2016. Polychlorinated biphenyls and polybrominated biphenyls. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 107. Lyon, France. http://monographs.iarc.fr/ENG/Monographs/vol107/mono107.pdf.
International Labour Organization (ILO), no date. Globally Harmonized System for the Classification and Labelling of Chemicals. http://www.ilo.org/legacy/english/protection/safework/ghs/ghsfinal/index.htm, section 5 "Acute Toxicity: Oral, Inhalation, Dermal".
International Union of Pure and Applied Chemistry (IUPAC), 2014. Compendium of Chemical Terminology, second ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). XML on-line corrected version: http://goldbook.iupac.org (2006-) created by M. Nic, J. Jirat, B. Kosata; updates compiled by A. Jenkins. ISBN 0-9678550-9-8. doi: 10.1351/goldbook. Last update: 2014-02-24; version: 2.3.3. DOI of this term: doi:10.1351/goldbook.AT06800 (direct URL https://goldbook.iupac.org/AT06800.html).
Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2002. Fifty-seventh Report, Evaluation of Certain Food Additives and Contaminants. In: WHO Technical Report Series, vol. 909. World Health Organization, Geneva, pp. 121–149.
Masuda, Y., 2001. Fate of PCDF/PCB congeners and change of clinical symptoms in patients with Yusho PCB poisoning for 30 years. Chemosphere 43, 925–930.
Medical Dictionary for the Health Professions and Nursing, Farlex 2012. Short-term Exposure Limit. Retrieved 31 Jul. 2016. http://medical-dictionary.thefreedictionary.com/short-term+exposure+limit.
Merck, 2017a. Safety Data Sheet for Acetone According to Regulation (EC) No. 1907/2006. Revision Date 26.05.2017 Version 19.4. http://www.merckmillipore.com/INTERSHOP/web/WFS/Merck-DE-Site/de_DE/-/EUR/ProcessMSDS-Start?PlainSKU=MDA_CHEM-822251&Origin=SERP.
Merck, 2017b. Safety Data Sheet for Ethyl Acetate According to Regulation (EC) No. 1907/2006. Revision Date 28.05.2017 Version 3.3. http://www.merckmillipore.com/INTERSHOP/web/WFS/Merck-DE-Site/de_DE/-/EUR/ProcessMSDS-Start?PlainSKU=MDA_CHEM-100789&Origin=PDP.
Mocarelli, P., Gerthoux, P.M., Brambilla, P., Marocchi, A., Beretta, C., Bertona, M., Cazzaniga, M., Colombo, L., Crespi, C., Ferrari, E., Limonta, G., Sarto, C., Signorini, S., Tramacere, P.L., 1999. Dioxin health effects on humans twenty years after Seveso: a summary. In: Ballarin-Denti, A., Bertazzi, P.A., Facchetti, S., Mocarelli, P. (Eds.), Chemistry, Man, and Environment: the Seveso Accident 20 Years on Monitoring, Epidemiology and Remediation. Elsevier Science, pp. 41–51.
Roth, 2016. Safety Data Sheet for Acetone ≥99.5 %, for Synthesis According to Regulation (EC) No. 1907/2006 (REACH), Amended by 2015/830/EU date of compilation: 2015-10-13, Revision: 2016-04-28. https://www.carlroth.com/downloads/sdb/en/5/SDB_5025_IE_EN.pdf.
Roth, 2015. Safety Data Sheet for Acetic Acid Ethyl Ester >99.5 %, for Synthesis According to Regulation (EC) No. 1907/2006 (REACH), Amended by 2015/830/EU date of compilation: 2015-11-12. https://www.carlroth.com/downloads/sdb/en/7/SDB_7338_GB_EN.pdf.
Scientific Committee on Food (European Commission, Health & Consumer Protection Directorate-General, Directorate C - Scientific Opinions), 2001. Opinion of the Scientific Committee on Food on the Risk Assessment of Dioxins and Dioxin-like PCBs in Food adopted on 30 May 2001.
ScienceLab.com, 2013a. Material Safety Data Sheet – Acetone MSDS. http://www.sciencelab.com/msds.php?msdsId=9927062.
ScienceLab.com, 2013b. Material Safety Data Sheet – Ethyl Acetate MSDS. http://www.sciencelab.com/msds.php?msdsId=9927165.
Sigma-Aldrich, 2015a. Sicherheitsdatenblatt zu „2,3,7,8-Tetrachlorodibenzo-p-dioxin solution" gemäß Verordnung (EG) Nr 453/2010, Version 5.6. überarbeitet am 29.12.2015.
Sigma-Aldrich, 2015b. Safety Data Sheet for Acetone According to Regulation (EC) No. 1907/2006, Version 5.5. Revision Date 14.01.2015.
Sigma-Aldrich, 2015c. Safety Data Sheet for Ethyl acetate according to Regulation (EC) No. 1907/2006 (Version 6.3 Revision Date 17.02.2015).
Signorini, S., Gertoux, P.M., Dassi, C., Cazzaniga, M., Brambilla, P., Vincoli, N., Mocarelli, P., 2000. Environmental exposure to dioxin: the Seveso experience. Andrologia 32, 263–270.
Sorg, O., Zennegg, M., Schmid, P., Fedosyuk, R., Valikhnovskyi, R., Gaide, O., Kniazevych, V., Saurat, J.H., 2009. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) Poisoning in Victor Yushchenko: Identification and Measurement of TCDD Metabolites. The Lancet. http://dx.doi.org/10.1016/S0140-6736(09)60912-0 published online August 5, 2009.
Starlab, no date. Gloves and Safety. http://starlab.de/int/index.php?l=2&action=products&section=0&catalognode=101260.
State of California, Division of Occupational Safety and Health (Cal/OSHA), no date. Permissible exposure limits for chemical contaminants. http://www.dir.ca.gov/title8/5155table_ac1.html.
Technische Regeln für Gefahrstoffe (TRGS 905), 2014. Ausgabe März 2014, GMBl 2014 S. 510–522 Vo. 19.5.2014. Ausschuss für Gefahrstoffe. www.baua.ags.
United States Department of Labor, Occupational Health & Safety Administration (OSHA), no date-a. Permissible Exposure Limits. https://www.osha.gov/dsg/annotated-pels/tablez-2.html.
United States Department of Labor, Occupational Health & Safety Administration (OSHA), no date-b. OSHA Regulations (Standards – 29 CFR), Part 1910-Occupational Safety and Health Standards, subpart Z – toxic and hazardous substances, 1910.1200 App A-Health Hazard Criteria.
United States Environmental Protection Agency (US EPA), no date. About Acute Exposure Guideline Levels (AEGLs). https://www.epa.gov/aegl/about-acute-exposure-guideline-levels-aegls.
United States Environmental Protection Agency (US EPA), 1994. Method 1613 Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, October 1994. https://www.epa.gov/sites/production/files/2015-08/documents/method_1613b_1994.pdf.
United States Environmental Protection Agency (US EPA), 2004. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) and Related Compounds National Academy Sciences (External Review Draft). EPA/600/P-00/001Cb.
United States Environmental Protection Agency (US EPA), 2012. EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments, vol. 1. EPA/600/R-10/038A.
Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World health organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93 (2), 223–241. http://dx.doi.org/10.1093/toxsci/kfl055.
VWR, no date. Leitfaden für Einweghandschuhe - VWRI9242. https://de.vwr.com/store/content/externalContentPage.jsp?path=/de.vwr.com/de_DE/vwr_handy_solution_guides.jsp.
Wellington Laboratories, 2013. Material Safety Data Sheet "2,3,7,8-Tetrachlorodibenzo-p-dioxin". Erstellungsdatum 01/01/2013.
World Health Organization (WHO), 2009. The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification. http://www.who.int/ipcs/publications/pesticides_hazard_2009.pdf.