Food and Chemical Toxicology 106 (2017) 574–582

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Current methods in risk assessment of genotoxic chemicals

Alexander Cartus*, Dieter Schrenk

University of Kaiserslautern, Food Chemistry and Toxicology, Erwin-Schroedinger-Strasse 52, 67663, Kaiserslautern, Germany

ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form
6 September 2016
Accepted 8 September 2016
Available online 13 September 2016

Keywords:
Risk assessment
Genotoxicity testing
Contaminants
Residues
Carcinogenicity
Genotoxicity

ABSTRACT

Chemical contaminants and residues are undesired chemicals occurring in consumer products such as food and drugs, at the workplace and in the environment, i.e. in air, soil and water. These compounds can be detected even at very low concentrations and lead frequently to considerable concerns among consumers and in the media. Thus it is a major challenge for modern toxicology to provide transparent and versatile tools for the risk assessment of such compounds in particular with respect to human health. Well-known examples of toxic contaminants are dioxins or mercury (in the environment), mycotoxins (from infections by molds) or acrylamide (from thermal treatment of food). The process of toxicological risk assessment of such chemicals is based on i) the knowledge of their contents in food, air, water etc., ii) the routes and extent of exposure of humans, iii) the toxicological properties of the compound, and, iv) its mode(s) of action. In this process quantitative dose-response relationships, usually in experimental animals, are of outstanding importance. For a successful risk assessment, in particular of genotoxic chemicals, several conditions and models such as the Margin of Exposure (MoE) approach or the Threshold of Toxicological Concern (TTC) concept exist, which will be discussed.

1. Introduction

Unwanted compounds or impurities of toxicological concern in consumer products such as food or pharmaceutical drugs can be divided into contaminants and residues (Codex Alimentarius Commission, 2015).

Contaminants are compounds which are found in environmental samples and food etc. Per definition, contaminants were not released or added intentionally to consumer products. Their occurrence usually is the consequence of insufficient prevention and/or cleaning precautions related to thermal or other chemical processes and/or of other inadequate production, handling or manufacturing of chemicals or during processes. Examples in food and feed are dioxins, mycotoxins and pyrrolizidine alkaloids (most frequently due to co-harvesting) or compounds formed during the production process such as acrylamide and ethyl carbamate. An exception, i.e., an intended introduction of a contaminant may occur for criminal purposes such as the illegal 'disposal' of contaminated materials in the feed chain of livestock. Examples are aniline (Spanish toxic oil syndrome) or polychlorinated biphenyls (PCBs) and dioxins which can enter the food chain *via* contaminated oil (Diggle, 2001; Bernard et al., 2002).

Besides contaminants there are **residues** present in consumer products. These are derived from the intentional use during the production process, mainly during plant protection (e.g. from the use of pesticides) or from the use of veterinary drugs etc. during agricultural production of animal products such as meat, fish, eggs or milk. Such compounds may also occur as contaminants as long as they originate from intentional use for such purposes but contaminate secondary targets such as soils or surface water (e.g. from the use of contaminated manure or from drift during plant protection measures).

However, this rigid classification cannot be applied strictly to all chemicals of toxicological concern in consumer products. Examples are plant-derived compounds such as alkenylbenzenes (e.g. estragole, methyleugenol) in herbs and spices or coumarin in cinnamon which are usually not regarded as contaminants because they are naturally occurring constituents in certain plants. Furthermore, 'genotoxic impurities' in pharmaceutical drugs may be introduced or arise during the synthesis of active ingredients and can be considered as contaminants (reaction side products) or residues (residual reagents such as heavy-metal catalysts, solvents or starting material; Szekely et al., 2015).

The distinction between contaminants and residues has extensive consequences for the risk assessment of these classes. Residues in a consumer product result from their intended use. Therefore, in

* Corresponding author.
E-mail address: cartus@rhrk.uni-kl.de (A. Cartus).

http://dx.doi.org/10.1016/j.fct.2016.09.012
0278-6915/

most cases such compounds have to go through a thorough legislative approval process where a risk assessment for the compound is made with regard for the purpose of use. For example, an active substance in a plant protection product has to be evaluated and approved ("positive list"). This requires a comprehensive toxicological characterization of the compound to make sure that no (or a justifiable) risk will emanate from the use of the compound (in the final product/formulation). The manufacturer of such a chemical is responsible to provide sufficient toxicological data as a prerequisite for the legal authorization.

In contrast, contaminants enter consumer products unintentionally. Therefore, the structural identification of contaminants, as well as the synthesis or production of sufficient amounts of "new contaminants" needed for toxicological tests can be an obstacle for the further toxicological characterization in many cases. The lack of detailed chemical and toxicological information of a certain contaminant concerns all steps of risk assessment and is hence a major challenge illustrating the need for alternative methodologies beside the classical approaches.

This paper will focus on food-borne toxicants with special emphasis but not exclusively on non-genotoxic and genotoxic carcinogens. Certain points concerning genotoxicity test methodologies, regulations and implications for the risk assessment are presented in more detail in the other articles being part of this Special Issue.

2. Utilization of data in toxicological risk assessment

Toxicological risk assessment of chemicals typically consists of four steps:

1. **Hazard identification** (i.e. determination of substances that may have inherently adverse effects under certain conditions of exposure).
2. **Hazard characterization** (i.e. the qualitative and desirably quantitative description of the nature of the hazard, such as toxicokinetics, mechanisms of action and dose-response relationships).
3. **Exposure assessment** (addressing the quantitative question of how much of a certain substance a defined population will be exposed to) and
4. **Risk characterization** (consolidation of evidence, reasoning, and conclusions gained and collected in steps 1–3. and the estimation of the probability of the occurrence of an adverse effect in a certain population, taking several uncertainties into account).

2.1. Hazard identification and characterization

Toxicological risk assessment of a compound starts with a detailed description of its chemical structure and the physicochemical properties. Furthermore, the pathways of formation or synthesis and the methods for quantitative analysis of the compound should be documented and discussed.

In the next step, the toxicologically relevant facts about the behavior and effects of the compound in biological systems (*in vivo* and/or *in vitro*) of interest are collected. Studies using *in vivo*-models are also designed to obtain information about the bio-, pharmaco- or toxicokinetic of the compound. This includes data related to the absorption from the gastro-intestinal tract, lung, skin etc., metabolism, distribution in the organism and elimination. Of course, the intact organism is the best model to gain such data. However, *in vitro* models at various levels exist, which may allow estimates for biokinetic parameters describing the aforementioned processes.

The next step in risk assessment is the thorough investigation of the adverse effects elicited by the compound. Biological systems to study many toxicological endpoints mainly comprise higher organisms such as experimental animals (most frequently mice and rats) and organs, cells, subcellular fractions or purified target molecules (e.g. enzymes) isolated thereof or even more complex 'organ- or human-on-a-chip' approaches (An et al., 2015), often in combination with *in silico*-calculations, for example *via* physiologically based biokinetic modeling (Sung et al., 2014; Abaci and Shuler, 2015; Lin et al., 2016). Since toxicity studies in humans cannot be carried out for both ethical and legal reasons (with the exception of testing mild effects on the skin etc.), also human primary cells, subcellular organelles (e.g. mitochondria or microsomes), enzymes, receptors etc. are studied. Furthermore, studies in established human or other mammalian, so-called permanent cell lines are common. *In vitro* investigations can play an important role in defining a mode of action (MoA) of a compound and are often initiated by findings from animal experiments. Frequently, primary cells isolated from animal or human tissues are used for this purpose. These show the advantage of being relatively similar to the cells *in situ* of the organism reflecting the responses of the latter towards chemical challenges. Unfortunately, such cells loose many of their cell- or tissue-specific properties after having been kept in culture for hours, days or weeks. Thus, animals must be sacrificed regularly in order to isolate fresh primary cells. Cell lines are widely used and a well alternative to *in vivo* experiments for several investigations. Once established in a laboratory, their availability is nearly unlimited, handling is comparatively easy and cheap. However, they often have very few specific properties in common with the parent cells *in situ*, which make them less useful if the knowledge about the toxicological properties of a compound is still low. Moreover, cell lines change their properties further once being transferred and kept over several passages. This leads to the situation that 'the same' cell line can differ when propagated over several cell generations in different laboratories which may limit their usefulness for toxicological research sometimes. However, progress has been made in this regard using 2D- or 3D (co-)culture models for many organs such as liver or skin and for certain types of cancer (Alépée et al., 2014; Nath and Devi, 2016). Taken together the predictive power of such *in vitro* methods is frequently overestimated with respect to adverse effects in humans and animals. However, such methods can be highly valuable, if a well-defined mechanistic hypothesis needs to be tested. For this purpose, the *in vitro* models should be as well characterized as possible. Examples are agonistic or antagonistic effects at certain receptors, the induction of apoptosis, cell division etc.

Again, one of the most reliable sources for toxicological data are animal experiments or, if available, observations in humans. Only these systems can provide information of reactions in a system as complex as the intact organism. Animal experiments are mostly carried out as acute, sub-acute or chronic feeding or treatment studies where the test compound is applied *via* the relevant route, for example oral (i.e. in the feed, the drinking water or per gavage) or per inhalation. Other application routes, for example (sub) cutaneous or intravenous administration are considered less relevant unless a compound is suspected to enter the body *via* such a route which may be relevant for a contaminant in a medical preparation intended for injection or dermal application etc. Particularly valuable if not compulsory for quantitative risk assessment is the use of various dose levels. Among those doses, at least one should elicit clear effects whereas other should be without (adverse) effects (Bucher, 2002; Rhomberg et al., 2007). Furthermore, a statistically sufficient number of animals should be used. All relevant findings and observations must be documented