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Threshold and non-threshold chemical carcinogens: A survey of the present regulatory landscape

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ABSTRACT

For the proper regulation of a carcinogenic material it is necessary to fully understand its mode of action, and in particular whether it demonstrates a threshold of effect. This paper explores our present understanding of carcinogenicity and the mechanisms underlying the carcinogenic response. The concepts of genotoxic and non-genotoxic and threshold and non-threshold carcinogens are fully described. We provide summary tables of the types of cancer considered to be associated with exposure to a number of carcinogens and the available evidence relating to whether carcinogenicity occurs through a threshold or non-threshold mechanism. In light of these observations we consider how different regulatory bodies approach the question of chemical carcinogenesis, looking in particular at the definitions and methodologies used to derive Occupational Exposure Levels (OELs) for carcinogens. We conclude that unless proper differentiation is made between threshold and non-threshold carcinogens, inappropriate risk management measures may be put in place - and lead also to difficulties in translating carcinogenicity research findings into appropriate health policies. We recommend that clear differentiation between threshold and non-threshold carcinogens should be made by all expert groups and regulatory bodies dealing with carcinogen classification and risk assessment.

1. Introduction

Appropriate regulation of a carcinogenic substance necessitates a full understanding of its mode of action, and in particular whether it demonstrates a threshold of effect. Insufficient attention to the presence or not of a threshold can lead to possible errors and inconsistencies in the way that a carcinogenic material is regulated. Identification of the potential carcinogenic hazard of a substance has traditionally been based on findings from a 2-year animal study following OECD guidelines. Such studies, often utilising rats and/or mice, are designed to detect potential induction of neoplastic lesions by a specific agent, in addition to providing information on target organ(s) and mode of action, determining dose-response relationships, and establishing a point of departure (POD) for non-neoplastic effects (EFSA, 2005). A number of alternative approaches of shorter duration (less than 2 years) have also been developed investigating endpoints relevant to cancer formation, e.g. induction of pre-neoplastic foci (EFSA, 2005).

Although carcinogens have also been identified from observations of tumour incidence in exposed human populations, these are limited to a few substances and generally involve high exposures and very specific tumour response (EFSA, 2005).

Following confirmation of carcinogenicity, whether in humans or animals, it is usual to determine whether the chemical (in its initial form and/or after undergoing metabolic changes) is genotoxic. This is normally achieved through a number of *in vitro* studies exploring mutagenicity in bacterial and mammalian cells and identifying 'indicator effects' such as DNA damage (strand breaks or adduct formation) or induction of DNA repair. Further *in vivo* studies are usually carried out to confirm any findings from *in vitro* assays (EFSA, 2005).

The current convention for defining carcinogens is to divide them into two categories according to the presumed mode of action: i.e. **genotoxic** and **non-genotoxic carcinogens**.

There are numerous potential modes of action for non-genotoxic carcinogens, all involving mechanisms other than genotoxicity. It is generally assumed that for non-genotoxic carcinogens – *but not for genotoxic substances* – a 'no-effect level' can be defined, i.e. that there is a threshold dose below which toxicity does not occur, although it is now increasingly accepted that genotoxic

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carcinogens may also have a demonstrable threshold of effect. The importance of a clear understanding of, and distinction between, threshold and non-threshold effects in carcinogenesis is deemed essential for the proper classification and regulation of carcinogenic substances.

A key focus of this paper is to explore the different approaches to this issue taken by a number of authoritative regulatory bodies, in particular in relation to the setting of occupational exposure limits. The concepts of genotoxic and non-genotoxic carcinogens and threshold and non-threshold carcinogens are investigated and recommendations made. Attention is given to chemicals found in the workplace and/or the environment; the paper does not include consideration of pharmaceuticals or radiation.

2. Genotoxicity

Genotoxic carcinogens are usually identified on the basis of positive results in different *in vitro* and *in vivo* test systems, including detection of DNA strand-breaks, unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE), DNA adduct formation and mitotic recombination (EFSA, 2005).

Tests for mutagenicity – typically the Ames test, *in vitro* metaphase chromosome aberration assay, *in vitro* micronucleus assay and the mouse lymphoma L5178Y cell Tk (thymidine kinase) gene mutation assay) - often precede (or accompany) such genotoxicity assays (ECHA, 2016) and generally constitute the first step in the hazard/risk assessment process. They are intended to provide only an indication of potential genotoxicity and, by extension, carcinogenicity.

Genotoxic carcinogens are categorised as direct or indirect acting, depending on whether there is interaction with DNA or inhibition of DNA synthesis, for example (Gillespie et al., 2011).

The mechanism by which a carcinogen mediates a genotoxic effect is considered the most important factor in determining the nature of the dose response curve, i.e., linear versus non-linear (O'Brien et al., 2006) and whether a threshold of effect exists. For direct acting genotoxic carcinogens, it has traditionally been assumed that a linear non-thresholded dose-response relationship exists, i.e. there is no dose below which cancer initiation does not take place because the mode of action may involve a single direct reaction; specifically, a single hit at a single target (Kirsch-Volders et al., 2000 – cited in Gillespie et al., 2012). In contrast, threshold-based mechanisms are conceivable for indirect acting genotoxic carcinogens (EFSA, 2005).

However, it is now increasingly accepted that both indirect *and* direct acting genotoxic carcinogens can show a non-linear (i.e. supra-linear or sub-linear) dose response, and may occasionally be truly thresholded. Therefore, in some cases, the default assumption of a linear dose-response for genotoxicity (and so for carcinogenicity) may not be justified (Greim and Albertini, 2015). The Scientific Committee of EFSA has concluded '*that based on current understanding of cancer biology, there are levels of exposure to substances that are both DNA-reactive genotoxic and carcinogenic, below which the cancer incidence is not increased (biological thresholds)*'. These arguments are recognised by DG SANCO of the European Commission and the US EPA, for example. Whilst not elaborating on the different types of carcinogens, ECHA recognises that threshold carcinogens exist and may be designated a NOAEL, and therefore a DNEL, as long as the mechanism of action is well defined (ECHA, 2012).

2.1. Threshold or non-threshold

It is accepted that non-genotoxic carcinogens have a conventional dose-response that allows identification of a threshold dose (e.g. NOAEL or LOAEL). Addition of uncertainty factors allows derivation of permissible exposure levels (PEL) at which it is anticipated that no relevant human cancer risks are anticipated. Examples of known non-genotoxic carcinogens include:

- Endocrine modifiers (e.g. 17-oestradiol). (Chen et al., 2008 - cited in Hernandez et al., 2009).
- Peroxisome proliferators (e.g. trichloroethylene). (IARC, 2014).
- Receptor mediators (e.g. 2,3,7,8-Tetrachlorodibenzodioxin (TCDD). (Whitlock, 1993 – cited in Hernandez et al., 2009).
- Immunosupressants (e.g. cyclosporine). (Buell et al., 2005 – cited in Hernandez et al., 2009).
- Inflammatory response initiators (e.g. metals such as vanadium and beryllium). (Ress et al., 2003 – cited in Hernandez et al., 2009).

For genotoxic carcinogens there are certain arguments, based on the mode of action, which may justify the derivation of a threshold dose (Streffer et al., 2004). For example, genotoxic carcinogens that give positive results in chromosomal assays in the absence of mutagenicity, e.g. aneugenicity or clastogenicity, may indicate the potential for a 'practical threshold' (Crebelli, 2000; Parry, 2000). Genotoxicity may also only be apparent under conditions of sustained local tissue damage and associated increased cell proliferation. A practical threshold can also be considered under these conditions. A key mechanism here is the production of reactive oxygen species (ROS) as high internal doses or high levels of ROS stimuli are clearly genotoxic (Bolt et al., 2004).

While a linear dose-response without a threshold is established for some carcinogens, for many others the precise dose-response at low doses has not been established; for these a linear dose response is assumed as the most precautionary approach (Streffer et al., 2004). However, as neatly illustrated by Bailey et al. (2009), linear extrapolation at low doses can result in marked over-estimation of cancer risk.

For genotoxic carcinogens, a diversity of methods must be considered when estimating carcinogenic risk at low doses, to reflect differences in modes of action (Bolt et al., 2004). In line with this, Streffer et al. (2004) has proposed four groups of carcinogens that should be tackled differently, as detailed below (examples given in Table 1):

- Non-threshold genotoxic carcinogens for which the ***linear non-threshold (LNT) model appears appropriate*** for low-dose risk assessment. Regulations may be based on the ALARA principle ("as low as reasonably achievable"), technical feasibility, and other socio-political considerations.
- Genotoxic carcinogens for which the existence of a ***threshold cannot be sufficiently supported.*** In these cases the linear-non-threshold model is used as a default assumption, based on the precautionary principle.
- Genotoxic carcinogens for which a ***practical threshold*** is supported by studies on mechanisms and/or toxicokinetics. Health-based exposure limits may be based on an established NOAEL.
- Non-genotoxic carcinogens and non DNA-reactive carcinogens. For these compounds a ***perfect threshold is associated with a NOAEL***, and health-based exposure limits can be derived.

Mutation induction is considered to be the key indicator of a direct, DNA reactive mutagenic mode of action and is the earliest key event in tumour development (Preston and Williams, 2005). Normal cellular function is continually threatened by DNA damage arising from a number of intrinsic and extrinsic sources; however several mechanisms exist to counteract potential insult to DNA including repair or removal of DNA damage. Mild DNA damage is