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Commentary

Chemical carcinogenicity revisited 2: Current knowledge of carcinogenesis shows that categorization as a carcinogen or non-carcinogen is not scientifically credible

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ABSTRACT

Developments in the understanding of the etiology of cancer have undermined the 1970s concept that chemicals are either "carcinogens" or "non-carcinogens". The capacity to induce cancer should not be classified in an inflexible binary manner as present (carcinogen) or absent (non-carcinogen). Chemicals may induce cancer by three categories of mode of action: direct interaction with DNA or DNA replication including DNA repair and epigenetics; receptor-mediated induction of cell division; and non-specific induction of cell division. The long-term rodent bioassay is neither appropriate nor efficient to evaluate carcinogenic potential for humans and to inform risk management decisions. It is of questionable predicitiveness, expensive, time consuming, and uses hundreds of animals. Although it has been embedded in practice for over 50 years, it has only been used to evaluate less than 5% of chemicals that are in use. Furthermore, it is not reproducible because of the probabilisitic nature of the process it is evaluating combined with dose limiting toxicity, dose selection, and study design. The modes of action that lead to the induction of tumors are already considered under other hazardous property categories in classification (Mutagenicity/Genotoxicity and Target Organ Toxicity); a separate category for Carcinogenicity is not required and provides no additional public health protection.

1. Introduction

Developments in the understanding of the etiology of cancer have profound implications for the way the carcinogenicity of chemicals should be addressed. This paper is one of three: the first paper (Wolf et al ., 2019) chronicles the history of carcinogenicity research and asserts that DNA coding errors that arise either through mutagenesis or cell proliferation lead to tumors; this second paper explains why the concept of carcinogens and non-carcinogens and the two-year bioassay are obsolete and unnecessary; and the third paper (Cohen et al., 2019) describes an animal-sparing, cost-effective testing plan for carcinogenic potential and potency.

1.1. Carcinogen or non-carcinogen categorization is fundamentally flawed

Classification for carcinogenicity is part of many regulatory schemes, *e.g.*, International Agency for Research on Cancer (IARC, 2015), United Nations Global Harmonised Scheme (UN, 2012) and the European Union Classification, Labelling and Packaging (ECHA, 2012). The use of these schemes has been criticised as no longer relevant (Boobis et al., 2016). These approaches are based on identification of carcinogenic *hazard* alone, not on an assessment of carcinogenic *risk* potential; thus, chemicals with up to 100 million-fold differences in their potency or likelihood to cause a cancer (CPDB, 2017) have been grouped in the same category. They grade the reliability of the evidence

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for carcinogenicity not the carcinogenic potential.

Research into the mechanism of action of known human chemical carcinogens revealed a link between mutagenesis and carcinogenesis (Ames, 1979). This link was initially supported by the results of 2-year bioassays in rodents; in these an increased incidence of tumors in rats and/or mice was observed when the animals were dosed for up to 2 years with known human carcinogens that had also been shown to be mutagenic. However, as more previously uncharacterized chemicals were tested in the 2-year bioassay, concern surfaced over the high proportion (about 50%) that caused an increase in the number of tumors, including many that were not mutagenic (Gold et al., 1989; Ames and Gold, 1990). These results began to call into question the value of the bioassay to accurately distinguish between chemicals that could cause cancer under real-world circumstances and those that would not.

In the absence of robust epidemiological data, the final arbiter of whether a chemical is considered to be a carcinogen or not has been based on the outcome of long-term rodent bioassays. The classification is an inflexible binary method.

If there is considered to be a treatment-related increase in neoplasms in any long-term rodent bioassay, the chemical is considered to be a carcinogen (unless there is sufficient mode of action evidence that indicate otherwise).

If there is considered to be no treatment-related increase in neoplasms in any long-term rodent bioassay, the chemical is considered to be a non-carcinogen.

This approach is incompatible with the current knowledge of the etiology of cancer (Wolf et al., 2019). Chemicals can increase the probability of cancer occurring by either direct interaction with DNA or enhancement of cellular proliferation. In either case, sustained exposure to the chemical results in an increased probability of cancer developing. The probability of inducing cancer is proportional to the potency of the chemical in exerting its effect, to the dose at which it is administered, to its toxicokinetic characteristics, and to the duration of dosing. The chemical is affecting the probability of events that are already taking place. Salsburg (1989) argued that it should be expected that many chemicals should affect the incidence of tumors in long-term bioassays as chemicals dosed at levels which cause a biological effect would disturb the “biochemical milieu” of the animal and this would change the pattern of lesions seen, including the number and type of tumors. The incidence could be increased or decreased. Salsburg (1983) noted that in over 20% of long term bioassays the incidence of tumors was significantly reduced. Salsburg posed the question as to whether reductions in incidence should be considered to have as much significance as increases, argung that it made no sense to divide the universe of chemiclas up into “carcinogens” and “non-carcinogens”.

Goodman and Wilson (1991) also recognised this issue and they came up with a radical alternative. They said it was unhelpful to classify chemicals as carcinogens or non-carcinogens, and they suggested that all chemicals could be assumed to be carcinogenic with some chemicals having a potency too low to produce a statistically significant increase in tumors in a given experimental situation. This idea is incorrect, as many chemicals do not have a mode of action which will lead to tumor formation however large the experimental dose. However, there is no bright line between carcinogens and non-carcinogens but rather there is a continuum with some chemicals having high potential, some having no potential, and others having potential at a point along the continuum. This continuum exists alongside other adverse effects which may show their effects at doses below those which may lead to the induction of cancer.

The implication for cancer prevention is not about whether a chemical will induce cancers in an experiment dosing rats or mice with a high dose for their lifetimes, but rather under what circumstances will it increase the incidence of cancer in humans. The carcinogenic potential must be defined by potency and take into account the modes/mechanisms of action as this will determine the measures that will be necessary to prevent cancer in humans.

1.2. Carcinogenic potential

Carcinogenic potential describes the ability of a chemical to induce cancer over a range of dose levels and exposure routes and durations. Estimates of carcinogenic potency have been derived from long term studies in the form of TD_{50}, TD_{25} or BMD_{10} [1]; in these estimates of potency the duration of exposure is fixed at two years which is assumed to represent a lifetime in the tested species, and, by extrapolation, to a human lifetime. This allows comparisons to be made between chemicals, but it does not allow estimates of the effects of different durations of exposure to be made.

Cohen and Ellwein (1990) used mathematical models to draw general conclusions about carcinogenic potential. Chemicals (or their active metabolites) that act via direct interaction with DNA such as 2-acetylaminofluorene (metabolite), diethylnitrosamine, dimethylnitrosamine and N-{4-(5-nitro2-furyl)-2-dithiazol]formamide) can induce cancer as a result of exposure earlier in the process because they cause mutations which can be carried forward in dividing cells. They are generally assumed not to exhibit a threshold. However, there is accumulating evidence that this may not be the case and there have been international workshops on quantitative dose response assessment for genotoxicity which have indicated that there are practical thresholds (MacGregor et al (2015a, 2015b)).

Chemicals that induce cellular proliferation without directly acting with DNA can be divided into two categories according to whether or not they interact directly with a cellular receptor. For those that act by a cellular receptor (such as phorbol esters, dioxins, and hormones), dose levels which do not activate the receptor will not trigger cellular proliferation and there will be no carcinogenicity, i.e. there will be a threshold for a carcinogenic response. Chemicals that act via a non-receptor mechanism such as cytotoxicity (for example chloroform) do not induce tumors at dose levels which are below the level at which the cytoxicity can occur. Tumors which result from non-genotoxic mechanisms are in reality a manifestation of chronic toxicity.[2] Some chemicals will cause neither genotoxicity nor proliferation/cytotoxicity, and will have no carcinogenic potential.

Carcinogenic potential, thus, can be defined as a function of the dose level and duration of dosing required to cause an increase in neoplasia via responses such as genotoxicity, cytotoxicity and cell proliferation. The lower the dose level and the shorter the duration of dosing that results in neoplasia, the higher the carcinogenic potential. More importantly, exposure at levels and durations that do not increase those responses will not cause an increase in cancer.

1.3. Interpretation of long term bioassays

Long term rodent bioassays have been used extensively for about a half century as the means to determine whether a chemical should be considered to be a human “carcinogen” or a “non-carcinogen”. These studies are typically designed to maximize the possibility of eliciting a tumorigenic response and as such will use the maximum limits of the dose level and duration. Although hazard only classification schemes do not convey chemical potency, an indication of the carcinogenic potency can be determined from the bioassay, *i.e.,* the dose range at which neoplasms may be induced. The potency of carcinogens covers a wide range; the range of TD_{50} values across chemicals that have induced

[1] TD_x: for a given target site(s), if there are no tumors in control animals, then TD_x is that chronic dose-rate in mg/kg body wt/day which would induce tumors in x% of the test animals at the end of a standard lifespan for the species. BMD_x: derived bench mark dose assumed to give x% of the animals tumors at a specific tissue after correction for spontaneous incidence, within the life time of that species.

[2] Adverse reaction of an organism to a continuous or repeated exposure to a chemical substance over a long period of time (NAL, 2015).

neoplasms in rodents is more than 100 million-fold (CPDB, 2017). Chemicals with high carcinogenic potential are likely to cause neoplasms in two-year bioassays, and chemicals with no carcinogenic potential are not likely to cause neoplasms.

However, chemicals with low or intermediate carcinogenic potential may or may not cause neoplasms in long term rodent bioassays, dependent on the conditions of the bioassay and the doses used and not, simply, as an inherent property of the chemical. First of all, the process of carcinogenesis is a multi-stage probabilistic process which means it will have high inherent variability. This implies that the reproducibility across cancer bioassays would be expected to be difficult to attain. This was demonstrated to be the case by Gottmann et al. (2005) who compared 121 replicate rodent carcinogenicity assays from the two parts (i.e. from the National Cancer Institute/National Toxicology Program and from the open literature) of the Carcinogenic Potency Database (CPDB) to estimate the reliability of these experiments. They estimated a concordance of only 57% between the overall rodent carcinogenicity classifications from both sources, just 7% better than flipping a coin. They also examined the quantitative relationship for carcinogenic potency where there was concordance for carcinogenicity and discovered a similarly low correlation between TD_{50} values ($r^2 = 0.63$).

Another source of variability is the way doses are set for long term rodent bioassays. The highest dose employed is the maximum tolerated dose (MTD), the highest dose which will not be expected to shorten the lifespan of the animals or cause greater than a 10% decrement in body weight (Rhomberg et al., 2007). This dose is dependent on the potency or the capability of the chemical to cause any toxicity. If the toxic potency is high, then the MTD will be low; if the toxic potency is low, then the dose needed to cause an effect will have to be very high. Most chemicals exert more than one adverse effect; for example, one mode of action may cause dose-limiting toxicity and another may cause toxicity leading to tumors. If the dose-limiting toxic effect occurs at lower doses than the tumorigenic effect, then insufficient chemical will be administered to induce tumors in the long-term bioassay and *vice versa*. Thus, two chemicals with the same carcinogenic potential could be classified differently depending on the relationship between the dose limiting toxicity and the carcinogenic mode of action. If the dose limiting toxicity and the tumorigenic toxicity occur at similar dose levels, then tumors may or may not result.

Another important variable, not only related to the dose but also the mode of action that leads to the tumor outcome and the particular type of tumor, is the duration of exposure. It is rarely sufficient to expose the rodent for a short-term to induce non-gentoxic tumors; for most chemicals there must be repeated long-term exposure for the lesions to develop and progress to tumor.

Despite the issues around the rodent bioassay, it is possible to draw some meaningful conclusions from the results of long term rodent bioassays. However, we know now that the conclusions are more nuanced than simply “carcinogen or non-carcinogen”.

- Chemicals that induce tumors in two-year bioassays have genotoxic or cellular proliferation (receptor or non-specific) activity at doses equal to or lower than the doses that induce the toxicity limiting the amount of chemical which can be tolerated.
- Chemicals that do not induce tumors in two-year bioassays either have no genotoxic or cellular proliferation (receptor or non-specific) activity or such activity occurs at doses higher than the doses which induce the toxicity which limits the amount of chemical which can be tolerated.

1.4. Alternatives to long term bioassays

Although the long-term rodent bioassay has been considered to be such a key study in assessing the toxicity of a chemical, relatively few chemicals have been assessed with this tool. There are approximately 50,000 chemicals in use commercially (Fischetti, 2010), but the databases for long term bioassays contain studies for only around 1500 (c.3%) chemicals (CPDB, 2017). The number has been limited by long-term rodent bioassays being expensive, time consuming, using large numbers of animals and other limitations coupled with the absence in most cases of a regulatory requirement. Today there are more appropriate means of achieving information to determine the cancer potential in humans. The trend in toxicology is to promote hypothesis-based testing that draws on an array of information including molecular methods. Consequently, there has been a drive to find alternative methods to assess carcinogenic potential which are more predictive, less expensive, quicker, and use fewer or no animals.

One problem, which has made such an effort difficult, is being stuck in the old practice of wishing to reproduce the binary “carcinogen/non-carcinogen” results of the long-term bioassay rather than move to a new paradigm in assessing the chemical's position on the spectrum of carcinogenic potential. The binary classification can usually be achieved with chemicals that interact directly with DNA. These chemicals often have high carcinogenic potential and generally give rise to positive results in both long-term bioassays and in short term *in vitro* and *in vivo* genotoxicity assays.

Not surpisingly given the limitations of the 2 year bioassay previously outlined, it has proved to be much more difficult to reproduce the results of long term rodent bioassays with non-genotoxic chemicals (Gottmann et al., 2005). Attempts to develop alternative assays that predict the results of long term rodent bioassays in terms of non-genotoxic “carcinogen/non-carcinogen” are, therefore, likely to be unsuccessful. An example of such an attempt is the European Union 6th Framework Programme funded collaborative study, carcinoGENOMICS, which explored a range of *in vitro* cell culture models for identifying organotypical genotoxic- and non-genotoxic hepatocarcinogen-specific gene signatures. The non-genotoxic carcinogens acted through various processes such as endocrine modification, immune suppression, inhibition of gap junction inter-cellular communication and apoptosis, epigenetic modifications, tissue-specific toxicity, and general inflammatory/stress responses. Analysis of the data at the gene and the pathway level by using independent biostatistical approaches showed a distinct separation of genotoxic from non-genotoxic hepatocarcinogens and non-carcinogens. The assay, however, could not discriminate between non-genotoxic “carcinogens and non-carcinogens” (Doktorova et al., 2014).

Another approach has been described by Smith et al. (2016) who put forward the concept of the key characteristics of carcinogens (KCs). They have analysed the properties shown by chemicals that have been classified as carcinogens and determined that they show one or more of 10 key characteristics. However, Smith et al. (2016) and Guyton et al. (2018) did not analyse the incidence of one or more KCs in chemicals that did not induce tumors, so the value of their analysis is limited. In addition, they did not attempt any quantitative analysis of the relationship between the identified characteristics and the dose-response of the *in vivo* carcinogenic effect.

The KCs describe an event or events which could play a role in a range of adverse effects, including, but not solely, the induction of tumors. The mere presence of one or more KC cannot lead to the arbitrary classification as a “carcinogen”.

If the current theory of carcinogenicity is correct, then it should be possible to detect toxicity in short-term studies which could lead to tumors in the long term. There have been investigations into how well the results of 13-week, 6-month and 12-month toxicity studies in rats predict the outcome of two-year bioassays in rats (Allen et al., 2004; Boobis et al., 2009; Jacobs, 2005; Reddy et al., 2010). Overall, these investigations indicate that the absence of hyperplasia, cellular hypertrophy, and atypical cellular foci in any organ or tissue in the shorter-term studies is a good predictor of lack of tumor induction in the two-year bioassay. The additional 6 months to 12 month studies did not improve the level of prediction. However, findings of cellular proliferation in the 3 shorter-term studies are not always correlated with

tumor induction in the two-year bioassay. This is not surprising given the low concordance between two-year bioassays on the same chemical reported by Gottmann et al. (2005); if replicated two-year bioassays are not predictive of each other, it is unreasonable to expect shorter assays to be any better. However, all of these findings, including the KCs, are consistent with the induction of tumors being a multi-step, probabilistic process with high variability.

The ability of shorter-term assays to predict lack of tumor induction in two-year bioassays is being systematically evaluated by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH, 2017) as a basis for waiving the requirement for a two-year rat bioassay in order to save animals, time, and resources. Pharmaceutical compounds are evaluated to assess their genotoxicity, pharmacological mode of action, and repeat dose toxicity. The process relies on high quality assays for genotoxicity, short term toxicity and cellular proliferation and one aim of the evaluation is to determine the reliability of these assays. At the end of this evaluation, compounds are placed into one of three categories:

Category 1 Highly likely to be carcinogenic in humans, such that rodent carcinogenicity studies would not add value.
Category 2 Uncertain carcinogenic potential, such that rodent carcinogenicity studies are likely to add value.
Category 3a Highly likely to be carcinogenic in rats through prior established and well recognised mechanisms known to be human irrelevant, such that a rat carcinogenicity study would not add value.
Category 3b Highly unlikely to be carcinogenic in both rats and humans, such that a rat carcinogenicity study would not add value.

Compounds that are genotoxic *in vivo* are placed into category 1, and a two-year bioassay is not required. Compounds that show no evidence of genotoxicity, no preneoplasia in any organ in a 90 day rat study, or hormonal perturbation are placed into category 3b, and a two-year bioassay is not required. Compounds showing only evidence of potential carcinogenicity by a mode of action known to be non-relevant to humans are placed into category 3a, and a two-year bioassay is not required. Compounds that do not meet these criteria are placed into category 2 and will have a two-year bioassay performed with the aim of reducing uncertainty concerning carcinogenic hazard.

The ICH process is a step in the right direction. It identifies compounds with high carcinogenic potential (Category 1), although compounds showing genotoxicity *in vivo* may not always lead to tumors in the bioassay for a variety of reasons such as DNA repair, or the genotoxicity being secondary to cytotoxicity. It identifies compounds with low carcinogenic potential (Category 3a and 3b). Unfortunately, the process then tries to force compounds with low to intermediate carcinogenic potential (Category 2) into the historical binary division of "carcinogen and non-carcinogen". It uses the two-year bioassay as a final arbiter of these categories; however, as we have argued in a previous section, the two-year bioassay has such high variability (because of the variability of the carcinogenic process it is trying to measure and the interplay between dose limiting toxicity and cell proliferation inducing toxicity) that the outcome of the assay for compounds with low to intermediate carcinogenic potential is little more than a lottery.

ICH requires two-year bioassay studies for Category 2 compounds in order to add value, but the actual added value is unclear. Does the value depend on how the compounds are subsequently managed? Are the risk management processes for the winners of the lottery (non-carcinogens) different from the risk management processes of the losers (carcinogens)? In practice, the risks are managed in the same way for pharmaceuticals. There are many pharmaceuticals that have been used safely for many years for which tumors have been observed in a two-year bioassay (Cohen et al 2019). The human therapeutic dose levels are set so that the beneficial effects are enabled but the toxic effects that led to the tumors are avoided. There are also many pharmaceuticals that can cause the same toxic effects, but produce no tumors in the two-year bioassay. These are also administered therapeutically at levels that avoid these effects.

On the other hand, some schemes applied to commodity chemicals and to crop protection products can result in very different outcomes for chemicals which lose the lottery. For instance in the EU, crop protection chemicals may be classified as carcinogens and subject to prohibition in use without a risk assessment process, although the risk to be managed is the same for lottery winners and losers – they have properties which may lead to tumors after prolonged, high dosing but preventing the effects prevents the tumors.

Braakhuis et al. (2018) examined the hypothesis that protection against effects seen in 90-day rat studies would also protect against tumorigenicity as detected in 2-year rat carcinogenicity studies of 44 chemicals. They compared the 90-day NOAEL (No-Adverse-Effect-Level) to the tumor NOAEL and the tumor bench mark dose calculated for 10% incidence of tumors (BMD_{10}) and for 1% incidence of tumors (BMD_{01}). They found that the 90-day NOAEL and the tumor NOAEL were similar. They also showed that the tumor NOAEL was similar to the tumor BMD_{01}. They concluded that there is justification for the current practice in European chemical regulation Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) of not asking for a 2-year rat study for chemicals that show no evidence of genotoxicity. Establishing the health-based guidance value for the chemical on the results of the rat 90-day study would protect against tumors.

1.5. Implications for classification

Risk management is the process of deciding whether and how to manage risks. Risk assessment provides information on potential human health or ecological risks, and risk management is the action taken based on consideration of that and other information. Risk assessment is based on hazard characterisation (what hazards are possible and under what conditions of dose magnitude and duration), and exposure assessment. Classification is a truncated version of hazard characterisation and should include both identifying the hazard and then placing the chemical into categories based on the degree of hazard. Categorisation on the basis of degree of hazard has been omitted from some classification schemes, such as IARC and GHS, and this has caused controversy (Boobis et al., 2016).

The aim of classification and labelling is to identify the hazardous properties of a substance or a mixture by applying specific criteria to the available hazard data (classification), and then to provide any appropriate hazard labelling and information on safety measures (ECHA, 2012). The current view of the etiology of cancer suggests that it is not useful to consider carcinogenicity as a single hazardous property with its own hazard category. The modes of action that can lead to the induction of tumors can be considered under other hazardous property categories. Direct interaction with DNA falls within the hazardous category Mutagenicity/Genotoxicity, and appropriate hazard labelling and information on safety measures are available for these properties. Receptor-mediated and non-receptor-mediated increases in cellular proliferation are toxic effects with a variety of adverse outcomes and they fall within the hazardous category Specific Target Organ Toxicity – Repeat Exposure. Appropriate hazard labelling and information on safety measures are available. Therefore, a separate hazardous category for Carcinogenicity is not necessary and, as argued, misleading and highly uncertain for non-genotoxic compounds.

2. Discussion

A basic understanding of carcinogenesis has evolved over the last half century, and several conclusions have been established (should be Wolf et al 2019):

1) Cancer is due to mistakes occurring in the DNA (usually in somatic cells, but can be inherited through germ cells).
2) More than one mistake in the DNA is necessary.
3) All of the mistakes need to accumulate in a single cell (clonal origin of cancer).
4) The cell population at risk are the tissue pluripotent (stem) cells.
5) Every time DNA replicates, permanent mistakes could occur.
6) Carcinogenesis is a stochastic process.

These conclusions provide the framework for the arguments put forward in this paper that the long-term rodent bioassay is not a valid method to evaluate carcinogenic potential. It is of questionable predicitiveness, expensive, time consuming, and uses hundreds of animals. After half a century, it has only been used to evaluate less than 5% of chemicals that are in use. It is not reproducible because of the probabalistic nature of the process it is evaluating combined with dose limiting toxicity, dose selection, and study design.

A range of *in vitro* and shorter-term *in vivo* assays can be used to evaluate carcinogenic potential (Cohen et al 2019). The aim of these assays should not be the pointless task of reproducing the results of long-term rodent bioassays in order to brand chemicals as carcinogens or non-carcinogens. Rather the aim should be to identify effects which may lead directly or indirectly to DNA changes or damage, or increases in cell division. Health is then protected by setting exposure limit values which would prevent occurrence of such primary effects. This would protect against all adverse long term effects, including cancer.

The modes of action that lead to the induction of tumors are already considered under other hazardous property categories in classification (Mutagenicity/Genotoxicity and Target Organ Toxicity); a separate category for Carcinogenicity is not required and provides no additional public health protection.

An assessment scheme based on this approach will allow human health to be safeguarded and far more chemicals to be fully evaluated, while eliminating a costly, outmoded and unnecessary assay.

Declaration of interests

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The authors' affiliations are as shown on the cover page. The authors had sole responsibility for the writing and content of the paper. The views and opinions expressed in the paper are those of the authors, and do not necessarily reflect the views or policies of the authors' current or former employers.

The authors have served as members of the following panels or committees and/or for the following organizations:

Council of Canadian Academies (VD); European Food Safety Authority (AB, AM); European Centre for Ecotoxicology and Toxicology of Chemicals (AB, JD); EU Scientific Committee on Occupational Exposure Limit Values (AM); European Medicines Agency (AB); Health Canada (PF-C); International Agency for Research on Cancer (SC, DW); International Life Science Institute (AB, SC, JD, VD, PF-C, AM, JS, RS, DW); Joint WHO/FAO Meeting on Pesticides Residues (AB, VD, PF-C, AM); Italian Committee on Pesticides (AM); Joint WHO/FAO Expert Committee on Food Additives (Residues of Veterinary Drugs) (AB); National Institutes of Health (SC); National Academy of Sciences (SC, PF-C, RS); National Institute of Environmental Health Sciences (SC, DW); National Toxicology Program (SC, VD, PF-C, JS, DW); Organization for Economic Cooperation and Development (VD, PF-C, JS, RS); Swiss Centre for Applied Human Toxicology (AB, AM); UK Advisory Committee on Pesticides (AB); United Kingdom Committee on Carcinogenicity (AB, JD); UK Committee on the Medical Effects of Air Pollutants (AB); UK Committee on Residues of Veterinary Drugs (AB); UK Committee on Toxicity (AB); United States Environmental Protection Agency (SC, VD, PF-C, JS, RS, DW); United States Food and Drug Administration (SC, PF-C); World Health Organization International Program on Chemical Safety (AB, SC, JD, VD, PF-C, AM, JS, RS).

VD, JS & RS are retired from the US Environmental Protection Agency.

PF-C is retired from the US Environmental Protection Agency and the International Life Sciences Institute.

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Appendix A. Supplementary data

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