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International Cadmium Association

Call for Evidence:

Occupational Exposure Limit for Cadmium and its Inorganic Compounds

PART 2

SUBMISSION OF INFORMATION ON HEALTH EFFECTS

MECHANISMS OF ACTION FOR THE GENOTOXIC ACTIVITY OF CADMIUM AND ITS INORGANIC COMPOUNDS

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Abstract

The differentiation between genotoxic and non-genotoxic carcinogens is an important element in carcinogenicity risk assessment, which leads to the use of different models for extrapolating low dose effects (linear non-threshold versus threshold approaches).

The present document aims at providing an updated literature summary on the genotoxicity data for cadmium and cadmium compounds, and at reaching a better understanding of the mechanisms of action involved in any associated genotoxic activity. It evaluates whether there is evidence to deviate from the default approach that assumes a non-threshold mechanism of the genotoxic/carcinogenic action.

The collected data support the consideration of cadmium as an indirect genotoxicant. *In vitro* genotoxicity data indicate negative mutation results in bacterial tests systems, generally positive data for *in vitro* mammalian gene mutations, chromosome aberrations, micronucleus and DNA damage induction. The *in vivo* genotoxicity studies confirmed the *in vitro* data while genotoxicity studies in exposed humans have shown mixed positive and negative findings.

The genotoxicity of cadmium occurs through mechanisms that indirectly cause damage to DNA or chromosomes, via interactions with peptides and proteins. Different mechanisms of action are involved: induction of oxidative stress, inhibition of DNA repair, deregulation of cell proliferation, interaction with apoptotic pathways and interaction with epigenetic mechanisms.

The various lines of evidence described in this document are consistent with a mechanism of action-based threshold for the genotoxic activity of cadmium and cadmium compounds. *In vitro* data demonstrate sufficient evidence that threshold dose-response relationships take place. Lowest concentrations inducing statistically significant effects could be identified with consistency across all genotoxicity endpoints. *In vivo* genotoxicity studies are reinforcing this evidence. The evidence from human workers data supports a threshold genotoxic mode of action as well.

1. Introduction

Genotoxic events are crucial steps in the pathogenesis of cancer. Genotoxicity assays are performed to provide information on the potential of chemicals to induce cancer or cause heritable damage in humans (ECHA, 2017). Genotoxicity data and the type(s) of genotoxic damage induced are useful for the determination of a general mode of action for a substance, can provide some indication on the dose (concentration)-response relationship, and on whether the observed effect can be reasonably assumed to have a threshold or not (ECHA, 2017).

This document has two objectives:

a) to provide an overview of the available genotoxicity data for cadmium compounds, as identified by a literature review run until December 2019. This overview mainly focuses on the literature published since the last SCOEL evaluation of cadmium and its inorganic compounds (SCOEL, 2017a);

b) to better understand the mechanisms of action involved in the genotoxic activity of Cd compounds, and to assess whether there is evidence to deviate from the default regulatory approach, which assumes a non-threshold mechanism of action.

For carcinogens like cadmium and its compounds (cadmium chloride, cadmium sulfate, cadmium nitrate, cadmium oxide, cadmium hydroxide, cadmium carbonate, cadmium sulfide), it is key to establish whether a threshold for the carcinogenic action can be identified (or not) as it will determine whether an OEL (or a cancer dose-response assessment) needs to be established (JTF, 2017).

Regulatory authorities generally distinguish between genotoxic and non-genotoxic carcinogens.

Non-genotoxic carcinogens act via other mechanisms than genotoxicity, e.g. tumour promotion, and it is is generally accepted that a threshold concentration exists below which these chemicals will not be carcinogenic (JTF, 2017; SCOEL, 2017b).

For genotoxic carcinogens two groups were identified by the RAC/SCOEL Joint Taskforce (2017), according to the mechanism of action (ECHA, 2019):

i. *"where genotoxicity is caused by **direct** interaction of the substance or its metabolite with the DNA, the risks are usually assessed using a linear dose-response relationship (non-threshold), unless substance-specific data are available that allow deviation from linearity and/or to derive a Mode of Action (MoA)-based OEL";*
ii. *"where genotoxicity may occur through **indirect** mechanisms that cause damage to DNA or chromosomes, frequently by interactions with proteins, and there is sufficient evidence that a threshold can be identified, then a MoA-based OEL may be derived."*

The starting (or default) assumption for genotoxic carcinogens is that there is no threshold for the carcinogenic hazard. When sufficient information is available, it may be possible to conclude on a threshold-based mode of the carcinogenic action (ECHA, 2019).

Metals act via indirect mechanisms of genotoxicity. Typical mechanisms activated by metals include the induction of oxidative stress, inhibition of DNA repair, activation of mitogenic signalling, and epigenetic modification of gene expression (Beyersmann et al., 2008). These mechanisms are non-stochastic in nature, and are characterized by a threshold type dose-response relationship.

The aim of this review is thus to assess whether the available evidence for cadmium and its compounds supports the idea that these substances act primarily via indirect mechanisms, allowing the derivation of a mechanism-based threshold for genotoxicity (and by extension for carcinogenicity).

2. Overview of the genotoxicity data

The genotoxicity of cadmium compounds has been reviewed by several organizations including IARC (1993, 2012), ATSDR (2012), EU RAR (2007) and SCOEL (2017a).

This paper includes data discussed in these reviews but also refers to papers published more recently, compiled in a literature review carried out by Professor G. Van Maele-Fabry and Professor D. Lison at the Louvain Centre for Toxicology and Applied Pharmacology (LTAP), UCLouvain. The list of recent papers that were apparently not included in previous evaluations (e.g. SCOEL 2017a) is attached as Annex.

The toxicity of metals usually involves the initial interaction of the free metal ion with the toxicological target (Hollenberg, 2010). Bioavailability of metal species, i.e. uptake through cell membranes, intracellular distribution and binding to cellular macromolecules, is a decisive factor in metal toxicity (Beyersmann et al., 2008). The toxicity of a metal species is mainly correlated to the free ion concentration at target organ-sites (Verougstraete, 2018). A review of the most common anions and their possible contribution to toxicity is available on the MISA blog hosted by Eurometaux (https://www.reach-metals.eu/misa-blog/documents/counterion-files-hh). The genotoxic properties of cadmium and its inorganic compounds are also related to the bioavailable divalent cadmium cations (Cd^{2+}). This paradigm has been applied in key reviews, including IARC, ATSDR and SCOEL. The contribution of the associated anions to the genotoxicity of Cd compounds is negligible. Table below shows grouping of the carcinogenic cadmium compounds into three categories based on their water solubility.

Cadmium compound	**Water solubility grouping**
Cadmium chloride	Very soluble
Cadmium sulfate	
Cadmium nitrate	
Cadmium hydroxide	Slightly soluble
Cadmium carbonate	
Cadmium oxide	
Cadmium metal	
Cadmium sulphide	Insoluble

Accordingly, the data below mainly include *in vitro* and *in vivo* genotoxic effects of cadmium chloride. This fully soluble Cd compound has been widely investigated in genotoxicity studies, and this extensive database is used here as a reference for other Cd compounds. The Cd chloride data will also be used to draw dose-relationships in section 3.2.

2.1. *In vitro* genotoxicity data

Gene mutations

Cadmium compounds (cadmium chloride, but also cadmium oxide) gave negative results in bacterial assays with *S. typhimurium* (Bruce and Heddle, 1979, Mortelmans et al., 1986).

In cultured mammalian cells, cadmium compounds generally showed positive results for gene mutations. Positive results were found in the *Hprt* gene mutation assays in Chinese hamster lung fibroblasts treated with cadmium chloride (Ochi and Ohsawa, 1983; Jianhua et al., 2006; Gobrecht et al., 2017). No or weak point *K-ras* mutation were observed in human lung fibroblasts treated with cadmium chloride or cadmium sulfate (Mouron et al., 2004). Cadmium sulfate showed a positive response in L5178Y mouse lymphoma cells at the thymidine kinase locus (Oberly et al., 1982).

Chromosomal aberrations/effects

Several studies have reported inductions of chromosome aberrations (CA), micronuclei (MN) and sister chromatid exchanges (SCE) for water-soluble and poorly water-soluble cadmium compounds. Cadmium chloride and, to a lesser extent cadmium sulfate, have been extensively studied.

Chromosome aberrations (CA)

Chromosomal aberrations following cadmium chloride exposure have been observed in mouse spleen cells (Fahmy and Aly, 2000), Chinese hamster ovary [CHO-W8] cells (Deaven and Campbell, 1980; Howard et al., 1991; Wang and Lee, 2001) and Chinese hamster skin, lung and kidney cells (Deaven and Campbell, 1980). Higher sensitivity of CHO cells as compared to other mammalian cell types has been reported, as well as influence of culture conditions on the results. A protective effect of foetal calf serum from cadmium damage, namely higher concentration levels needed for significant induction of CA, was clearly demonstrated by Wang and Lee (2001) and Deaven and Campbell (1980). Chromosomal aberrations observed *in vitro* were predominantly of chromatid type indicating that

DNA double strand breaks were not among the major damage induced by cadmium chloride (Wang and Lee, 2001).

Studies on human cells, including peripheral blood lymphocytes (Deknudt and Deminatti, 1978; Rozgaj et al., 2002, Gateva et al., 2013) and lung fibroblast cell line MRC-5 (Güerci et al., 2000) have shown mixed results. Deknudt and Deminiatti 1978 observed no significant increase of numerical or structural aberrations up to 50 µM $CdCl_2$. Ambiguous results were reported by Rozgaj et al. (2002) with increased CA (acentric fragments) at all tested concentrations (100, 1000 and 5000 µM $CdCl_2$) but without concentration-dependency and with important interindividual differences. Gateva et al. (2013) showed positive results with statistically significant increase in CA (isochromatid breaks predominantly) at all tested concentrations (1, 10, 50 and 100 µM $CdCl_2$) with clear concentration-dependent effects observed in 3 out of the 5 donors. In human lung fibroblast cell lines (MRC-5), increased frequency of aneuploid cells was observed at all tested concentrations (0, 1, 2, 4 µM $CdCl_2$) (Güerci et al., 2000).

To summarize, positive results were observed in mammalian cells studies starting from 0.8 µM $CdCl_2$ after 2h treatment in serum free medium (Wang and Lee, 2001). In human cell studies, positive results were reported starting from 1µM $CdCl_2$ (Güerci et al., 2000) (see table 1 in Annex).

Micronuclei (MN)

Positive results have generally been reported after cadmium chloride exposure for *in vitro* micronucleus induction in mammalian, human cell lines, or human peripheral blood lymphocytes.

Dose-dependent increased frequencies of MN were observed in V79 cells, statistical significance being reported from 3 µM and 10 µM $CdCl_2$ by Ustundag et al. (2014) and Gobrecht et al. (2017), respectively. In mouse lymphoma L5178Y cells (with and without cytokinesis block method), significant increases of micronucleated cells were observed at 0.25 and 0.40 µg/ml [1.36 and 2.18 µM $CdCl_2$], by Fellows and O'Donovan, 2010 and Lorge et al., 2010, respectively. Similar results were reported in Chinese hamster ovary cells (CHO cell line): marked increases of MN with and without cytochalasin B shown at 0.26 µg/ml [1.42 µM $CdCl_2$] (Whitwell et al. (2010).

Significant increases in MN were also observed in human cell lines starting from 0.5 µM $CdCl_2$ with cytochalasin B (HepG2 cells, Peng et al., 2015), from 1 µM with cytochalasin B (MRC-5cells, Seoane and Dulout, 2001), from 4 µg/ml [21.8 µM] with and without cytochalasin B (TK6 cells, Fowler et al., 2010) and from 20 µM with cytochalasin B (MG-63, Oliveira et al., 2014). Micronucleus assays performed with human lymphocytes showed positive results with dose-dependent increases of MN frequencies in three studies (Berces et al., 1993; Lewinska et al., 2008; Turkez et al., 2012). The lowest concentrations showing significant results were 1, 5 and 16.4 µM $CdCl_2$, respectively. Ambiguous

results were reported by Kasuba and Rozgaj (2002) and by Rozgaj et al. (2002). Kasuba and Rozgaj (2002) evaluated the genotoxic effect of $CdCl_2$ (1-1000 µM) when the chemical was added to cultures in the G_0 and S phases of the cell cycle using cytochalasin B blocked MN assay. The authors reported increases in MN changing with the phase of the cell cycle. A significant increase was observed only for phase S and inter-individual variability between the two donor samples was observed. In the study of Rozgaj et al. (2002), the MN assay gave significant results for the three tested donor samples at the highest concentration of $CdCl_2$ (5000 µM) while the results were inconsistent in lower concentrations (no correlation between $CdCl_2$ concentrations and MN frequencies; inter-individual variability).

Seoane and Dulout (2001) and Ustundag et al., 2014 tried to discriminate between clastogenic and aneugenic effects by kinetochore staining and by using CREST (anti-centromere) antibodies, respectively. Discordant results were reported. Seoane and Dulout (2001) showed that $CdCl_2$ induced statistically significant increases of kinetochore-positive (K+) and kinetochore-negative (K-) MN arising from aneugenic and clastogenic events, respectively, at the two highest concentrations (2 and 4 µM) but higher increases of K+ MN frequencies (arising from aneugenic events) were observed (borderline statistical significance starting at 1 µM). As the test only allows detection of malsegregation, the authors proposed that this mechanism is at least one of those by which $CdCl_2$ induced aneuploidy. The CREST analysis (Ustundag et al., 2014) revealed that MN induced by $CdCl_2$ were predominantly CREST-negative, indicating clastogenic activity. These effects are often associated with processes involving oxidative stress. Thus, micronuclei due to both aneugenic and clastogenic activity of $CdCl_2$ have been observed *in vitro*.

To summarize, the lowest effective concentration for induction of micronuclei was observed at 0.5 µM $CdCl_2$ after treatment in serum free medium (Peng et al., 2015). The lowest positive concentration in studies using media with serum was 1µM $CdCl_2$ (Seoane and Dulout, 2001) (see table 1 in Annex). Induction of MN by $CdCl_2$ may vary depending on the phase of the cell cycle, and both clastogenic and aneugenic effects have been reported.

Sister chromatid exchanges (SCE)

For the most part, *in vitro* studies have shown induction of sister chromatid exchanges by cadmium chloride. Interpretation of the relevance of both positive and negative results is however unclear.
Discordant results were observed in mouse spleen cells (Fahmy and Aly, 2000) and in Chinese hamster ovary (CHO-W8) cells (Deaven and Campbell, 1980, Howard et al, 1991, Wang and Lee, 2001).
Studies on human cells, including peripheral blood lymphocytes (Rozgaj et al., 2002; Turkez et al., 2012; Dirican and Turkez, 2014; Verma et al., 2019) and lung fibroblast cell line MRC-5 (Mouron et al.,

2004) have all shown positive results with significant increases starting from 1 µM $CdCl_2$ in MRC-5 cell lines (Mouron et al., 2004) and from 16.4 µM in peripheral blood lymphocytes (Turkez et al., 2012).

Based on the *in vitro* SCE data, the lowest effective concentration showing statistically significant increase of SCE was observed at 1 µM $CdCl_2$ (Mouron et al., 2004).

DNA damage

Several studies have reported on DNA damage for cadmium compounds. The overview on *in vitro* DNA damage below focuses on cadmium chloride as reference compound and discusses successively findings in mammalian and human cells (see table 1 in Annex and cadmium chloride data used in section 3.2. "$CdCl_2$ genotoxicity- *in vitro* dose -response relationships").
Cadmium chloride induced DNA damage (both single and double strand breaks) in somatic cells in the great majority of studies detected by the alkaline comet assay, the alkaline elution technique and the histone H2AX phosphorylation assay (reporter of DNA double strand breaks).

Comet assays performed in Chinese hamster lung fibroblasts (V79) cells reported concentration-dependent DNA damage (Jianhua et al., 2006; Ustundag et al., 2014; Gobrecht et al., 2017). Statistical significant increase in tail length (TL) were reported from 1 µM $CdCl_2$ by Jianhua et al. (2006), significant increases in tail moments (Gobrecht et al., 2017) and in tail intensity (Ustundag et al., 2014) were observed from from 2.5 µM and 5 µM, respectively. Concentration-dependent increases of single-strand breaks (SSB) and of double-strand breaks (DSB) were reported (Gobrecht et al., 2017). Li et al. (2017b) showed that Cd induced DNA damage via ROS generation. Comet assay performed in HT-22 hippocampal mouse cell line showed concentration-dependent increase of DNA damage, statistically significant from 3.26 µM $CdCl_2$ (Karri et al., 2018). Comet assay was negative in mouse embryonic fibroblast cell line (NIH3T3): no alteration of tail DNA percentages neither of tail moment were observed at 5 and 10 µM $CdCl_2$ (Chen et al., 2016). Robison et al. (1982) quantitated strand breaks by determining the average molecular weight of DNA following treatment with $CdCl_2$ and observed reductions of DNA molecular weight at 10 and 100 µM (no statistical analysis). Ochi and Ohsawa (1983) using the alkaline elution test in V79 cells observed concentration-dependent increase in SSB only in combination with protein kinase K digestion of cell lysates, indicating formation of DNA-protein cross-linking by the metal.

The comet assay has been applied to several types of human cells. Mouron et al. (2001) reported increased percentage of lung fibroblasts [MRC-5] with tails at the higher dose (4 µM $CdCl_2$). The same author showed inconsistent findings in 2004 in the same cell line: slow and not significant increment

in tail length and tail moment and significant but not concentration dependent increase in comet moment (Mouron et al., 2004). Positive results were observed in the three studies on human hepatoblastoma cells [HepG2] (Skipper et al., 2016; Li et al., 2017a; Lawal and Ellis, 2010). Skipper et al. (2016) showed significant concentration-dependent increases in SSB starting from 5.5 µM $CdCl_2$. Significant increases in tail DNA percentage and in DNA tail intensity were reported from 0.01 µM and from 10 µM, respectively (Li et al., 2017a; Lawal and Ellis, 2010). Significant increases in DNA tail intensity were observed by Lawal and Ellis, 2010 also in two other cells types (astrocytoma cell line [1321N1] and human embryonic kidney cells [HEK293]) at the highest tested concentration (50 µM $CdCl_2$). Positive results were also observed in others human cells. In colon carcinoma cells (SW480) significant increase of tail length was observed at the two higher concentrations starting from 67 µM $CdCl_2$ (Curcic et al., 2014). In osteoblast-like MG-63 cells significant increase in DNA fragmentation was observed at 20 and 50 µM $CdCl_2$ after 48h treatment but not after 24h treatment (Oliveira et al.,2014). Belliardo et al. (2018) showed significant increase of DNA strand breaks starting from 100 µM $CdCl_2$ in normal dermal cells. Concentration-dependent increases in DNA damage were observed from 5 and from 50 µM $CdCl_2$ in Jurkat T cells (Nemmiche et al., 2011) and in human peripheral blood lymphocytes (Verma et al., 2019), respectively. In another study performed in human peripheral blood lymphocytes (Rozgaj et al., 2002), ambiguous results were reported with notable inter-individual difference. Significant concentration-dependent induction of DNA DSB was reported, starting from 25 µM $CdCl_2$ in SQ20B (epithelium tumors of the larynx) (Trabelsi et al. 2016) and in HepG2 (hepatoblastoma cells) cells and from 50 µM $CdCl_2$ in LS-174T (epithelial colorectal adenomacarcinoma cells) cell lines (Kopp et al. 2018). Trabelsi et al. (2016) suggests that DBS are the main DNA damages induced by $CdCl_2$ in SQ20B cell line. The impact of oxidative DNA damage and nucleotide excision repair was studied in cultured A549 cells (Schwerdtle et al., 2010). Induction of oxidative DNA damage was investigated by the alkaline unwinding technique in combination with Fpg. A significant induction of DNA strand breaks and Fpg-sensitive sites was observed only at 75 µM $CdCl_2$, a strongly cytotoxic dose.

To summarize, DNA damage has been demonstrated at the lowest effective concentrations of 0.01µM $CdCl_2$ (Li et al 2017a) and 1µM $CdCl_2$ (Jianhua et al 2006). Both single and double DNA strand breaks have been reported.

2.2. *In vivo* genotoxicity data

The genotoxic potential of cadmium chloride in *in vivo* studies is described below and summarized in Table 2 in annex. In the list of reported *in vivo* data, the most recent data since the SCOEL 2017 assessment, are also included.

Gene mutations

In somatic cells

The study of Jianhua et al. (2006) showed that *Hprt* mutant frequencies in the lymphocytes of rats exposed to $CdCl_2$ by an intraperitoneal injection were statistically higher than in controls.

In germ cells

Cadmium chloride did not induce heritable germ cells mutations in the dominant lethal mutation assay after intraperitioneal injection (Epstein et al., 1972; Suter, 1975; Gilliavod and Leonard, 1975) and oral gavage (Sutou et al., 1980). However, the dominant lethal test is generally considered to be rather insensitive.

Chromosomal aberrations/effects

Chromosome aberrations

In somatic cells

Bone marrow chromosome aberrations tests conducted with $CdCl_2$ in Swiss mouse all reported dose-dependent statistically significant increases in chromosome aberrations (Fahmy and Ali, 2000, Mukherjee et al, 1988; El-Habit and Moneim, 2014). In Fahmy and Ali, 2000 and Mukherjee et al, 1988, the animals were treated by intraperitioneal injections, while in El-Habit and Moneim, 2014 the animals were treated by subcutaneous injections. The aberrations types were mainly structural aberrations of chromatid-types involving breaks and gaps, but fragments, exchanges, deletion and translocation were also reported. Numerical chromosome aberrations (aneuploidy) in the form of hyper or hypoploidy were seen by Fahmy and Ali (2000) and El-Habit and Moneim (2014) as well as endomitosis suggesting an effect on the mitotic spindle at different stages of cell cycle (Fahmy and Ali, 2000).

In germ cells

Numerical (most frequently) as well as structural chromosome aberrations were induced in male (Fahmy and Aly, 2000; Miller and Adler, 1992) and female (Watanabe et al, 1979, Watanabe et al 1982)

germ cells. Positive results were observed in mice after subcutaneous (Watanabe et al 1982) and intraperitoneal (Miller and Adler, 1992 and Fahmy and Aly, 2000) treatment with $CdCl_2$ and in Syrian hamster after subcutaneous treatment with $CdCl_2$ (Watanabe et al, 1979). The study of Mailhes et al. (1988) reported negative results on numerical chromosome aberrations in female mice treated by intraperitioneal injection with $CdCl_2$. Explanation proposed by these last authors may be differential sensitivity to aneuploidy induction depending on the preovulation time of $CdCl_2$ administration. Gilliavod and Leonard (1975) reported that treatment of male mice with cadmium chloride did not induce heritable chromosomal translocation in the F1 generation.

Micronuclei

In somatic cells

Significant and dose-dependent increases of erythrocyte micronuclei were reported in most studies after intraperitioneal, subcutaneous or oral administration of $CdCl_2$ in mouse and suckling rats. For most of the studies the route of administration was by intraperitoneal injection (Bruce and Heddle, 1979; Mukherjee et al., 1988; Jagetia and Adiga, 1994; Fahmy and Aly, 2000; Viswanadh et al., 2010). Cadmium chloride was subcutaneously injected in the study by El-Habit and Moneim (2014), orally administered by Celik et al. (2009) and orally and subcutaneously administered in the study of Kasuba et al. (2002). Positive results were observed in all studies except that of Bruce and Heddle (1979) in which no effect on the frequency of MN was reported in the bone marrow of female hybrid mice. Dose-dependent increased frequency of MN were observed in the positive studies (Fahmy and Aly, 2000; Viswanadh et al., 2010; Jagetia and Adiga, 1994; Mukherjee et al., 1988; El-Habit and Moneim, 2014) except for Celik et al. 2009 and Kasuba et al. (2002) where a single dose was administered orally and by subcutaneous injection respectively. Evidence of bone marrow toxicity, as determined by a significant decrease in the ratio of PCE/NCE (Jagetia and Adiga, 1994; El-Habit and Moneim, 2014; Viswanadh et al., 2010; Celik et al., 2009) or by a significant increase in the frequency of PCE as compared to the control (Fahmy and Aly, 2000), was reported from 0.25 mg/kg bw, 3.67 mg/kg bw, 7 mg/kg bw, 15 mg/kg bw and 5.7 mg $CdCl_2$/kg bw, respectively, confirming that the test substance reached the target cells. No significant effects on the PCE/NCE ratio were observed by Mukherjee et al. (1988) neither by Celik et al. (2009) in peripheral blood erythrocytes.

Sister chromatid exchanges

In somatic cells

Potential induction of sister chromatid exchange by $CdCl_2$ injected intraperitoneally was studied *in vivo* in male Swiss mouse by Fahmy and Aly (2000) and by Mukherjee et al (1998). Both studies reported dose-dependent significant increase in number of SCE per cell.

DNA damage

In somatic cells

There is evidence from several mouse/rat studies that $CdCl_2$ can induce DNA damage in several organs and blood, after inhalation, oral, subcutaneous or ip administration, as detected by the alkaline comet assay (Valverde et al., 2000; Devi et al., 2001; Kasuba et al., 2002; Yang et al., 2012; Breton et al., 2013; Agnihotri et al., 2015; Wada et al., 2015; Ghosh and Indra, 2018) and alkaline unwinding assay (Saplakoglu et al., 1997). In addition to the classic comet assay, Breton et al. (2013) applied modified comet assay (FpG+) to detect oxidative DNA damage.

Valverde *et al.* (2000) investigated the induction of genotoxicity (as SSB and alkali-labile sites detected by the comet assay) in several organs of CD-1 mice after cadmium chloride inhalation (0.08µg/cc) . DNA damage was recorded after a single inhalation, but this was not increased by subsequent exposures. After single inhalation, the brain was the most sensitive organ while after 4 weeks exposure, the liver showed the highest DNA damage. The authors also reported correlation between length of exposure, DNA damage and Cd-tissue concentration. Some organs showed less damage than others and this could be as a consequence of the capacity to remove the damage induced by long periods of exposure, possibly because of the induction of detoxifying mechanisms such as induction of metallothionein.

In mice treated orally, dose-dependent significant increases of DNA damage were shown by several authors (Devi et al., 2001; Yang et al., 2012; Agnihotri et al. 2015): dose-dependent increase in tail length (TL) at 24h post-treatment (indicating DNA damage) and time-dependent decreases in TL at 48h (indicating DNA-repair) with $CdCl_2$ doses from 0.5 to 128 mg/kg bw (Devi et al., 2001); dose-dependent increases in TL, comet length (CL), tail moment (TM) and olive tail moment (OTM) in lungs from immature mice (Yang et al., 2012); dose-dependent increase in % of DNA in tail, significant from 1mg/L was reported by Agnihotri et al. (2015) in mouse blood from orbital vessels. Breton et al. (2013) showed bell-shaped curve of dose-effect relationship with significant increase of DNA fragmentation at 5 mg/L and no significant effect at higher doses in mice gastrointestinal tract (duodenum and colonic cells). By using the modified comet assay, the same authors showed no statistically significant increases of DNA fragmentation in duodenal cells and significant but not dose-related effect in the colonic cells. Significant increased % tail DNA were reported by Wada et al. (2015) in liver cells from 20 mg/kg/d and in stomach cells from 40 mg/kg/d but no dose-response linear trends were observed

and the authors of this study considered the results as equivocal. One single dose was used by Kasuba et al. (2002), Ghosh and Indra (2018) and Saplakoglu et al. (1997) which does not allow a dose-response analysis. Significant increases in % of tail DNA, tail length, tail moment and olive tail moment were reported in male rat cardiomyocytes after intragastric administration of 5 mg/kg bw/d $CdCl_2$ for 30 days (Ghosh and Indra, 2018). Significant increase in tail length was observed in reticulocytes after oral as well as after subcutaneous administration of 0.5 mg/kg bw $CdCl_2$ but no significant differences were observed in tail intensity (Kasuba et al., 2002). The study of Saplakoglu et al. (1997) investigated following single intraperitoneal injection of 4mg/kg $CdCl_2$, the the formation of DNA SSBs in rat lung and kidney. The amount of single-strand DNA breaks in rat kidney was much lower than in the lung (Saplakoglu et al., 1997).

In germ cells

DNA damage was measured in germ cells after $CdCl_2$ exposure by Nava-Hernandez et al. 2009 and Li et al., 2017b) by applying respectively the comet assay and assessing oxidative DNA damage by measuring 8-OH-dG level. Nava-Hernandez et al. (2009) reported dose-dependent increased % of DNA damage found in primary spermatocytes from rats administered orally (drinking water) during 13 weeks. Oxidative DNA damage of $CdCl_2$ was investigated after intraperitoneal injection of 2 mg/kg bw/d for 7 days and resulted in significant increased level of 8-OH-dG in testis of mice (Li et al., 2017b). No dose-response relationship could be established as only one dose was tested.

Overall, *in vivo* studies are mainly consistent with *in vitro* studies. Most studies performed in somatic cells detected gene mutations, chromosomal aberrations, micronucleus formation, sister chromatid exchanges and DNA damage. Also, in germ cells, significant inductions of chromosome aberrations and DNA damage were reported. No heritable germ cells mutations in the dominant lethal mutation assay were reported.

2.3. Human genotoxicity studies

No study on the mutagenic potential of cadmium in germ cells in humans is available.

To investigate the mutagenic potential of cadmium in somatic cells (peripheral blood lymphocytes) in humans, studies have been performed in workers from different occupational fields and in people living in cadmium-polluted areas. In those settings, it was not possible to isolate exposure to a single

Cd compound, and exposure to mixtures of Cd compounds was almost the rule. In some studies, an increase in the frequency of chromosome aberrations, micronuclei or sister chromatid exchanges was observed. However, from the evaluation of the studies in the EU RAR (2007), it is apparent that the studies are afflicted with shortcomings regarding study design, exposure assessments or consideration of confounding factors, limiting their value as evidence for a causal relationship between exposure to cadmium and genotoxicity. Since the 2007 EU risk assessment report, there have been several additional studies, but most of the limitations noted above remain relevant.

The SCOEL (2017a) assessment concluded, taking into account the data from the analysis of EU RAR (2007) and the ATDSR (2012), that human genotoxicity data are conflicting but are seemingly indicating a genotoxic potential for Cd and its compounds in occupational settings.

Data from human genotoxicity studies and occupational exposure to cadmium (workers, inhalation route) are reported in Table 3 and summarized below per endpoint. This current evaluation of the human genotoxicity data, including the most recent papers, focuses on human genotoxcity data since the SCOEL (2017a) evaluation.

Chromosomal aberrations/effects

Chromosome aberrations

Conflicting results on CA were observed. CA were investigated in several occupational settings including Zn industry (Deknudt et al., 1973), Cd plant (Deknudt and Leonard, 1975), alkaline battery factory (Bui et al., 1975), Cd-Zn smelting plant in Zn electrophoresis (Bauchinger et al., 1976), manufacture of Cd pigment (O'Riordan et al., 1978) and stabilizer production plants (Fleig et al., 1983), workers exposed to fumes and dusts in the production of Cd, Zn, Ag and Cu alloys (Forni et al., 1990) and workers in an electroplating factory (Abrahim et al., 2011). Co-exposure of the workers to several other metals, potentially genotoxic, makes it impossible to deduce which is responsible for the increased CA rates and in addition, between metals interferences may result in synergistic or antagonistic effects. Increased rates of CA were reported in 3 studies at B-Cd of at least 3.95 µg/l (Bauchinger et al., 1976; Abrahim et al., 2011) or of U-Cd higher than 10 µg/l (Forni et al., 1990; Abrahim et al., 2011). No study has shown correlation between CA induction and Cd occupational exposure.

The chromosomal aberrations reported were mostly structural abnormalities while few data on numerical abnormalities (aneuploidy).

Structural abnormalities: significantly increased percentages of cells with structural aberrations were observed in Exposed (E) versus Controls (C) in the studies of Bauchinger et al. (1976) and of Abrahim et al. (2011; except the chromatid and chromosome gaps). A significant increase was only shown for high intensity, long-term exposure by Forni et al. (1990). No significant difference in CA frequencies between E and C was reported by Bui et al. (1975), O'Riordan et al. (1978) and Fleig et al. (1983). Ambiguous unexplained findings were shown in the two studies from Deknudt and collaborators (Deknudt et al., 1973; Deknudt and Leonard, 1975): highest percentage of cells with structural anomalies was found in the subgroup with supposed low Cd (and Pb) exposure (Deknudt et al., 1973) and lowest mean percentage of cells with structural abnormalities was found in the subgroup with supposed high levels of Cd (and Pb) exposure but the incidence of more complex CA was significantly higher in this subgroup as compared to controls (Deknudt and Leonard, 1975). It has to be stressed that the exposed groups of these last studies included workers with signs of lead poisoning (saturnism).

Numerical abnormalities: significant increased tetraploïdy was reported by Abrahim et al., 2011, two studies scored only cells with a complete number of chromosomes (Deknudt et al., 1973; Bauchinger et al., 1976). Bui et al. (1975) reported loss or gain of chromosomes in a few cells probably due to technical factors at the chromosome preparation. Aneuploidy was not investigated in the remaining studies (Deknudt and Leonard, 1975; O'Riordan et al., 1978; Fleig et al., 1983; Forni 1994).

Overall, these controversial and/or unexplained questionable results do not allow to conclude on the potential of Cd compounds to increase the prevalence of CA.

Micronuclei

Conflicting results on MN were reported. MN were investigated in several occupational settings including production of Cd, Zn, Ag and Cu production factory (Forni 1994), metal powder-producing factory (Hamurcu et al., 2001), battery plant (Palus et al., 2003), harbor (Wegner et al., 2004), welders (Iarmarcovai et al., 2005), battery manufacture (Kasuba et al., 2010), ceramic industry (Kasuba et al., 2012) and manufacturing or recycling Ni-Cd batteries plants (Lison et al., 2019). All studies were cross-sectional studies except Wegner et al., 2004 which was a longitudinal study.

Statistically significant increased frequencies of MN in exposed vs controls were reported by Hamurcu et al. (2001); Palus et al. (2003); Iarmarcovai et al. (2005); Kasuba et al. (2010, 2012). Negative results were shown by Forni (1994), by Wegner et al. (2004) as well as by Lison et al. (2019) at median concentrations of 0.4 and 3.67 µg/g creatinine, respectively, for U-Cd and of 0.7 and 3.7 µg/l for B-Cd.

Exposure biomonitoring data did not provide plausible explanation for the discrepancy among the results, as low mean B-Cd levels (around 1.1 µg/l) resulted in increased MN mean values as compared to controls (Iarmarcovai et al., 2005; Hamurcu et al., 2001) and conversely negative MN findings were observed at higher B-Cd values (around 4-5 µg/l) by Forni (1994) and Lison et al. (2019). Positive (Iarmarcovai et al., 2005) and negative (Wegner et al., 2004) MN findings were reported for similar U-Cd concentrations (0.3 µg/g creatinine and 0.4 µg/g creatinine, respectively).

No study showing an increase in MN could establish a correlation with the exposure biomarkers except Kasuba et al. (2012). In this study, a significant association between B-Cd and MN was observed in a multivariate regression analysis. However, in this study, no significant difference in B-Cd was reported between exposed workers and controls. The lack of correlation between the level of MN in Cd-exposed workers and exposure level is of some concern. No rational explanation can be advanced to explain the observed discrepancies but co-exposure of the workers to several other potentially genotoxic metals, and the possible between-metals interferences can be sources of between studies differences.

Of particular interest is the observation from the recent study performed by Lison et al. (2019) who did not record a dose-response relationship for MN frequency in circulating lymphocytes of workers with a wide range of occupational exposure (up to 12.5 µg/l B-Cd and 20 µg/l U-Cd). This study has several strengths compared to previous works, including an a priori power calculation, a careful selection of healthy workers covering a large array of Cd exposure levels documented by seniority, B-Cd and U-Cd, the use of the CBMN (Cytochalasin-B MN) assay, the inclusion of positive controls, the documentation of and adjustment for potential confounders through multivariate statistical analyses.

Sister chromatid exchanges

Significant increase of sister chromatid exchange (SCE) in peripheral human lymphocytes associated with occupational exposure to Cd, was observed in workers in an electroplating factory in Egypt (Abrahim et al., 2011), in a battery plant in Poland (Palus et al., 2003) and in harbour workers in Germany (Wegner et al., 2004). In all studies exposure biomonitoring parameters (B-Cd and U-Cd) were higher in exposed groups as compared to controls.

B-Cd and U-Cd significantly correlated with SCE in two studies (Abrahim et al., 2011; Wegner et al., 2004) but not in the third (Palus et al., 2003). These last authors concluded that the lack of correlation between the level of genotoxic damage in Cd-exposed workers and exposure level is of some concern, but is probably due to the fact that their exposure measurements do not adequately reflect the integrated exposures of these workers over a longer period of time.

Mean/median values from studies showing positive results for SCE and for correlation analyses were between 0.7 and 18.66 µg/l for B-Cd and between 0.4 µg/g creatinine and 18.47 µg/l for U-Cd (Wegner et al., 2004 and Abrahim et al., 2011, respectively).

DNA damage

DNA damage was investigated in several occupational settings including facilities with high air-Cd expected (production of cadmium containing pigments and cadmium containing batteries, galvanization and recycling of electric tools) (Hengstler et al., 2003), battery plant (Palus et al., 2003), welders (Iarmarcovai et al., 2005; Botta et al., 2006), ceramic industry (Kasuba et al., 2012), goldsmiths (Moitra et al., 2015), metal workers (Sani and Abdullali, 2016) and traffic police wardens (Rashid et al., 2018). Redundancy exists between the data from Iarmarcovai et al. (2005) and Botta et al. (2006) issued from the same population. The alkaline comet assay was used to evaluate DNA damage in all studies except that of Hengstler et al. (2003) applying the alkaline elution method.

Cd exposure induced significant increased levels of DNA damage in the exposed workers in all studies as compared to Cd-unexposed workers whatever the measurement parameter used and the occupational setting.

Hengstler et al. (2003) reported that air-Cd and B-Cd influence the level of DNA-single strand break (SSB) and that co-exposure of Cd, cobalt and lead may cause more than multiplicative effects. According to the authors, the mechanism behind these interactions might be repair inhibition of oxidative DNA damage. Significantly increased DNA fragmentation (including SSB and alkali-labile sites) in lymphocytes was reported by Palus et al. (2003) followed by DNA repair after a 3h incubation of the cells. When two biological samples were taken from the workers at the beginning (BW) and at the end (EW) of a work week, the alkaline comet assay revealed that welders had a significant increase of olive tail moment chi-squared [OTMχ^2] distribution at EW compared to controls or to BW; no significant difference was found between BW and controls (Iarmarcovai et al., 2005; Botta et al., 2006). Significant difference in level of DNA damage was reported between metal workers in Nigeria and controls (Sani and Abdullali, 2016). These authors suggest that the comet effects could at least partly be attributable to traffic exposure in addition to metal fumes as the workers were likely exposed to higher levels of traffic air pollution than the controls. Similarly, significantly higher percentage of cells with DNA damage (Tail length) was observed in traffic police wardens of Pakistan (Rashid et al., 2018). Kasuba et al. (2012) evaluated DNA damage (measured by tail length, tail intensity and tail moment) in pottery-glaze workers and showed significantly higher values for all three parameters in exposed vs controls, with the best results for tail intensity. Comet assays were performed on the sputum cells to

determine the amount of DNA damage (Tail length) among Indian goldsmiths and showed higher amount of DNA breakdown among exposed vs controls (Moitra et al., 2015).

Correlation analyses showed a correlation between air-Cd (range: 0.05-138.00 µg/m^3) and DNA-single strand breaks (Hengstler et al., 2003). Conflicting results were reported for the analyses of correlation between DNA damage and B-Cd or U-Cd concentrations.

B-Cd was significantly correlated with levels of DNA damages in blood cells (Hengstler et al., 2003; Kasuba et al., 2012; Rashid et al., 2018) and in sputum cells (Moitra et al., 2015). No significant relationship was found between B-Cd and levels of DNA damages by Botta et al. (2006) and by Palus et al. (2003) nor between DNA damage and time of welding during the work week (Iarmarcovai et al., 2005). Divergent results were reported in the studies analyzing the correlation between U-Cd and DNA damage: significant association was reported by Moitra et al. (2015) but not by Botta et al. (2006). Sani and Abdullali (2016) showed a significant relationship between years of exposure and DNA damage.

3. Mechanisms for the induction of genetic damage by cadmium compounds

3.1. Review of the different modes of action associated with Cd compounds

IARC (2012) concluded that the genotoxicity of cadmium compounds mainly involves **indirect mechanisms** because cadmium salts do not cause DNA damage in cell extracts or with isolated DNA (Valverde et al., 2001). Interactions with peptides and proteins appear to be the most relevant genotoxic mechanisms, and several targets are identified, including antioxidant defense systems and DNA repair processes. Additional effects of Cd^{2+} ions on tumor suppressor and signal transduction proteins contribute to the carcinogenic activity of cadmium compounds (Hartwig 2010; 2013; 2018). This section will describe these different mechanisms based on available *in vitro* studies for $CdCl_2$ (2.1). We will then draw dose-response relationships (3.2), and discuss the consistency of *in vivo* and human epidemiological findings with a threshold mechanism (3.3).

1. *Induction of oxidative stress*

Unlike most carcinogenic metallic ions, Cd^{2+} ions are not able to participate in redox reactions under physiological conditions (Fenton-like reactions). Therefore, cadmium compounds are unable to

produce reactive oxygen species (ROS). However, Cd^{2+} ions can induce oxidative stress and lipid peroxidation, by depleting gluthathione and protein-bound sulfhydryl groups as well as through inhibition of antioxidant enzymes (i.e. superoxide dismutase, peroxidase and catalase) (Chen et al., 2019). Another possible mechanism, is the displacement of redox active transition metals, e.g . Fe^{2+} in metallothionein, indirectly giving rise to Fenton reactions (Hartwig, 2013; Filipic et al., 2012).

Experimental evidence supports that cadmium-induced genotoxic effects are mediated by oxidative stress. The involvement of oxidative stress in DNA damage has been reported in several *in vitro* studies using Chinese hamster lung fibroblasts (V79) (Jianhua et al., 2006; Ustundag et al., 2014), liver carcinoma cells (Skipper et al., 2016), human hepatoblastoma cells (Li et al., 2017a) and human peripheral blood lymphocytes (Verma et al., 2019), as examples. As shown and discussed in section 3.2. below, '$CdCl_2$ genotoxicity – *in vitro* dose-response relationships', these studies point towards thresholds for Cd-induced oxidative DNA damage.

The induction of DNA strand breaks and CA by cadmium in mammalian cells was suppressed by antioxidants and antioxidant enzymes, indicating the involvement of ROS and/or oxidative stress (Ochi et al. 1987; Stohs et al., 2001, Valko et al., 2006; reviewed in Beyersmann et al., 2008, Hartwig et al., 2013). Exposure of human lymphoblastoid cells to Cd resulted in the generation of 8-OHdG, a marker for oxidative DNA damage (Mikhailova et al., 1997; Filipic et al., 2012).

In addition to induction of DNA damage, ROS play a role in many other cellular and molecular events that have relevance to cadmium-induced genomic instability and carcinogenesis. Cadmium-induced oxidative stress induces overexpression of oncogenes *c-fos* and *c-jun*, regulated by the redox-sensitive AP-1 transcription factor (Filipic et al., 2012; Joseph et al., 2009). Furthermore**,** oxidative stress can cause genetic and epigenetic changes, uncontrolled cell growth, and abnormal cellular signaling (Luevano et al., 2014). Overall, all these events are non-stochastic in nature and thus expected to exhibit a threshold dose-response relationship.

2. Inhibition of DNA repair

Convincing evidence suggests that cadmium is capable to inhibit DNA repair, including mismatch, base excision and nucleotide excision repair. Lack of functional DNA repair allows for the accumulation of cells with DNA damage which, following cell division, will produce mutations and thus genomic instability, and, in turn, increased probability of developing cancer (Filipic, 2012; Chen et al., 2019).

In base-excision repair, low concentrations of cadmium, which do not generate oxidative damage as such, inhibit the repair of oxidative damage in mammalian cells (Dally and Hartwig 1997, Fatur et al., 2003). This has been observed by direct comparison in HeLa cells. While the induction of DNA strand

breaks by $CdCl_2$ was restricted to 10 µM or higher, the removal of oxidative DNA base modifications induced by visible light and recognized by the bacterial formamidopyrimidine (Fpg) was inhibited from 0.5 µM cadmium. A complete inhibition was observed at 5µM, a non-cytotoxic concentration in this test system (Daly and Hartwig, 1997). Jianhua et al. (2006) reported that the lowest concentration of $CdCl_2$ that can cause *hprt* locus mutations through inhibition of the DNA repair process was 0.1 µM (clone assay) while 1 µM can cause DNA damage (comet assay). Further evidence that DNA repair inhibition is a prominent mechanism in genomic instability induced by cadmium has been reported by Schwerdtle et al., 2010. In this study, both cadmium chloride and the largely water insoluble cadmium oxide inhibited the removal of benzo[a]pyrene induced DNA lesions. Concentration-dependent inhibition of DNA adducts repair started at non-cytotoxic concentration of 5 µM $CdCl_2$ and 0.2 µg/cm^2 CdO.

All these studies point towards thresholds for the *in vitro* impairment of DNA repair caused by cadmium compounds. This inhibition of DNA damage repair is due to the effect of Cd^{2+} ions on the enzymes and proteins that play key roles in the repair process. Several of these enzymes are members of the zinc finger family of proteins. Cd can be substituted for zinc in these enzymes, but the proteins with substituted Cd do not perform their functions as efficiently to repair DNA damage (Joseph et al., 2009). Such DNA repair inhibition mechanisms are concentration dependent and characterized by threshold dose-response relationships, e.g. to displace the physiological Zn^{2+} ions.

3. Dysregulation of cell proliferation and disturbance (inhibition) of tumor suppressor functions

Cadmium interacts with a multitude of cellular signal transduction pathways, many of them associated with mitogenic signaling (Beyersmann et al 2008). In various cell types *in vitro*, cadmium ions activate mitogenic protein kinases, transcription and translation factors, and induce the expression of cellular proto-oncogenes, *c-fos*, *c-myc* and *c-jun* (reviewed by Waisberg et al., 2003). In addition to directly stimulating mitogenic signals, cadmium also inhibits the control of cell proliferation. It inactivates the tumor suppressor protein p53 and inhibits the p53 response to damaged DNA (Méplan et al. 1999). Cadmium can affect cell cycle progression. Multiple reports focusing on cancer cell lines showed that Cd causes an arrest in the G1 level among the cell cycle. Oliveira et al. (2014) showed that Cd exposure induced a decrease in the percentage of cells in G0/G1 and an increase in the percentage of cells in S phase, suggesting a delay in S phase. In contrast, a normal proliferative cell cycle, especially in G1 or G2 phase, was reported by Trabelsi et al. (2016) in human head and neck cells (SQ20B cell line). These results could be explained by a molecular characteristic of the SQ20B cells resulting in functionally altered p53 expression (Trabelsi et al., 2016). Multiple interaction of cadmium ions with cell cycle

controls proteins, having Zn finger structures, are necessary to effectively dysregulate cell proliferation. Oliveira et al. (2014) also studied the expression of genes related to the cell cycle proteins and DNA damage checkpoints. The profile of disturbance of the cell cycle progression was associated with an increase of the levels of CCNE1 transcript levels for cells exposed to 50 µM of $CdCl_2$ after 24 and 48h. CCNE1 encodes for cyclin E1 which accumulates at G1/S boundary and is degraded as cell progresses to S phase. One of the main functions of CCNE1 is to activate CDK2 in the transition from G1/S phase. As reported below under section 'Gene expression', the study of Fischer et al. (2016) is indicating that the modulation of genes related to cell cycle regulation are mainly modulated at higher cadmium concentrations.

The above data on the dysregulation of cell proliferation and inhibition of tumor suppressor p53 by cadmium also indicate threshold dose-response relationships.

4. Interaction with apoptotic pathways

Cadmium has a dual effect on apoptosis. On the one hand, Cd inhibits apoptosis and on the other hand Cd induces apoptosis.

Several studies show that cadmium inhibits apoptosis induced by genotoxic agents. Cadmium has been reported as an inhibitor of apoptosis induced by other metallic and non-metallic toxic agents.

As an example, the study of Yuan et al. (2000) showed in Chinese hamster ovary (CHO) cells that $CdCl_2$ at concentrations ≥ 5 µM inhibited hexavalent chromium induced apoptosis in a concentration-dependent fashion (5-20 µM), possibly by inhibiting caspase-3, a central mediator of apoptosis.

There is also considerable evidence that cadmium induces apoptosis by caspase dependent and independent mechanisms involving mitochondria (for review Filipic, 2012). The *in vitro* genotoxicity data reported in section 2.1., demonstrated dose-dependent induction of apoptosis by cadmium in the range of low concentrations (lower than 10 µM) (Lewinska et al., 2008; Karri et al., 2018). Gobrecht et al. (2017) reported that induction of apoptosis in Chinese hamster lung fibroblasts (V79) occurred at low intracellular Cd concentration (12.9 ppb) (which corresponds to an extracellular concentration of 5µM $CdCl_2$) and resulted in the formation of SSB and DSB leading to mutations. The data demonstrated a concentration-dependent caspase-mediated induction of apoptosis.

Cadmium chloride-induced apoptosis was observed mainly at doses around 20 µM and higher (Oliveira et al., 2014; Skipper et al., 2016). From the study of Jianhua et al. (2006), it appears that clearance of DNA strand breakage induced by oxidative stress resulted from two major processes: DNA repair and apoptosis.

The modulation of apoptotic genes was also reported in several gene expression studies. The recent study by Fischer et al (2016) discussed below under 'Gene expression', is indicating that the modulation of apoptotic genes was more restricted to higher concentrations of cadmium in the respective cell lines investigated. A distinct activation of genes coding for the intrinsic signaling cascade indicative for mitochondrial damage was observed at the transcriptional level, characterized by the induction of the pro-apoptotic gene *PMAIP1* coding for NOXA, and a down-regulation of the anti-apoptotic *BCL2*. This pattern agrees with observations described previously for cadmium, disturbing the balance of anti-apoptotic and pro-apoptotic proteins of the Bcl-2 family [reviewed in Thevenod and Lee (2013)].

Apoptosis is regulated by receptor-mediated mechanisms and intracellular signalling cascades. The induction of apoptosis results from receptor–ligand and enzyme–substrate interactions. It therefore seems likely to assume that a certain definite number of such interactions will be required to elicit apoptosis although the number of molecules required for interactions to be effective may be very low in specific situations (Schulte-Hermann et al., 2000).

The above data indicate a threshold dose-response relationship for the induction or inhibition of apoptosis by cadmium.

5. Interaction with epigenetic mechanisms

Cadmium can interact with epigenetic mechanisms, including reversible epigenetic patterns of DNA methylation and histone modifications that regulate how genes are expressed (Filipic, 2012).

The study of Takiguchi et al. (2003) showed that following acute exposure (1 week) to cadmium chloride (up to 2.5 µM), TRL 1215 rat liver cells exhibited decreased DNA methyltransferase activity and global DNA hypomethylation, whereas extended exposure (10 week) resulted in increased DNA methyltransferase activity and DNA hypermethylation. A study using human prostate cells was in line with these results by showing that a 10-week exposure to cadmium led to malignant transformation in association with DNA hypermethylation at the global level, overexpression of DNMT3b and increased DNMT (DNA methyltransferase) activity, promoted hypermethylation and decreased expression of the tumor suppressor genes RASSF1A and p16INK4A (Benbrahim-Tallaa et al., 2007). Changes in DNA methylation are thought to have a tumor promoting effect, since it is associated with increased expression of cellular proto-oncogenes, and an increase of DNA methylation results in the silencing of tumor suppressor genes (Hartwig, 2010; IARC, 2013).

As reviewed by Venza et al. (2014), Ryu et al. (2015), very few studies have shown posttranslational modifications of N-terminal tails of histones after cadmium treatment. Acute exposure of nontransformed human urothelial cells (UROtsa) to cadmium markedly increased the expression of

enolase (ENO) 2, a useful biomarker for identifying tumoral neuroendocrine differentiation. It has been demonstrated that the expression of ENO2 mRNA was markedly elevated following treatment with the histone deacetlyase inhibitor MS-275 or in cadmium-and arsenicum-transformed UROtsa cells and tumour transplants (Soh et al., 2012). A similar mechanism has been proposed for MT-3 gene expression, but in reverse. MT-3 gene is silent either in cadmium or arsenicum-transformed cell lines derived from UROtsa cells (Somji et al., 2011) through a mechanism that implies histone changes. After treatment with MS-275, higher expression of MT-3 mRNA was found in the cadmium transformed cell lines as compared with the parental UROtsa cells. This demonstrates that Cd may block histone modifications through inhibition of histone modifying enzymes.

Epigenetic effects may contribute in an important way to the carcinogenic process, e.g., changes in methylation which can modify the expression of recessive mutations, changes in intercellular communications and all types of modulators of gene expression. These epigenetic targets support the idea that cancer frequencies might show threshold-like responses (Kirsch-Volders et al., 2000).

Permanent alterations in gene expression can arise from epigenetic effects on nucleoproteins. Because of the abundance of such proteins, numerous interactions over an extended duration are required to elicit an effect, which is not consistent with the "one hit" 'linear-no-threshold' model (Kobets et al., 2019).

The above data point towards epigenetic effects induced by cadmium, characterized by threshold dose-response relationships.

Changes in Gene Expression

Traditional *in vitro* genotoxicity assays (as discussed under 2.1.1.) are often time-consuming and have the limitations that they frequently lack the ability to provide mechanistic insight. Alternative approaches are gene expression analysis (transcriptomics) to assess the cellular response pathways activated after exposure.

The results from Fischer et al. (2016) on the gene expression analyses in cadmium-treated A549 adenocarcinoma (10 or 50 µM $CdCl_2$) and BEAS-2B epithelial bronchial (5 or 10 µM $CdCl_2$) cells for 8 or 24 hours are consistent with the above conclusions from the traditional *in vitro* genotoxicity assays on mechanisms for cadmium-induced genotoxicity.

Fischer et al. (2016) investigated the modulation of cellular signaling pathways with a high-throughput RT-qPCR analysis on BioMark™ HD System comprising 95 genes, covering important signaling pathways in maintaining genomic stability. The selected genes are grouped in different signaling pathways and cellular processes: redox-regulated transcription factors, proliferation and cell cycle

control, DNA damage response and repair, oxidative stress response, apoptosis and xenobiotic metabolism. A549 adenocarcinoma and BEAS-2B epithelial bronchial cells lines are both models for bronchial epithelial cells and have been selected since the lung is the major target organ of cadmium carcinogenicity upon inhalation exposure at workplaces and via smoking. Fischer et al (2016) demonstrated that cadmium activated genes were coding for the stress response, anti-oxidative defense, mitotic signaling and cell cycle control as well as the intrinsic apoptotic pathway. DNA damage response genes were induced but specific genes coding for DNA repair proteins involved in all major DNA repair proteins were downregulated. The results revealed distinct dose- and time-dependent, and cell type-specific gene expression patterns. Concerning dose-dependency patterns, the study showed that several groups of genes such as metallothionein (MT) and ROS and heat shock-sensitive genes (HMOX1 and HSPA1A) were regulated at both concentrations tested. In contrast, genes related to cell cycle regulation and apoptosis and DNA repair were mainly modulated at higher concentrations. Regarding time-dependent interactions, effects were most pronounced after 8 hours treatment, indicating an acute and strong reaction toward elevated intracellular cadmium ion levels, elevated generation of ROS and DNA damage. An obvious time dependent difference was seen in the modulation of the cell cycle regulation and proliferation genes. At 8h, both cell cycle arrest and proliferation-associated genes were affected, while at 24h only the proliferation stimulus was still visible. The gene expression effects were more pronounced in the non-tumorigenic, p53 deficient BEAS-2B cells in comparison to the A549 p53 proficient cancer cell line. Those differences in characteristics of cell lines (p53 status) are important factors when interpreting gene expression profiles.

Overall, the quantitative gene expression analysis reflected known interactions of cadmium related to its genotoxic and carcinogenic potential, namely the induction of metallothioneins, oxidative stress, DNA maintenance and tumor suppressor functions as well as modulations of cellular signaling (reviewed in Hartwig 2010, 2013). Thus, this approach provided a comprehensive overview on the interaction of cadmium with distinct signaling pathways, also reflecting molecular modes of action in cadmium-induced genotoxicity/carcinogenicity. It is clear that in interactions of Cd^{2+} ions with gene expression, multiple mechanism are operative. These mechanisms mediating the genotoxic and carcinogenic activity of Cd^{2+} ions are all non-stochastic in nature and thus expected to exhibit a threshold.

3.2. $CdCl_2$ genotoxicity – *in vitro* dose-response relationships

The mechanisms involved in the primary genotoxic activity of Cd compounds described above, reflect indirect genotoxic activities of the ionic Cd^{2+} species. Those indirect mechanisms and the *in vitro* genotoxicity data (section 2.1.) suggest the existence of a threshold dose-response relationship. No evidence is available that cadmium elicits secondary genotoxic mechanisms, involving genetic damage resulting from the oxidative DNA attack by reactive oxygen species generated by inflammation, but their contribution cannot be excluded mainly in the respiratory tract upon inhalation exposure (see figure below).

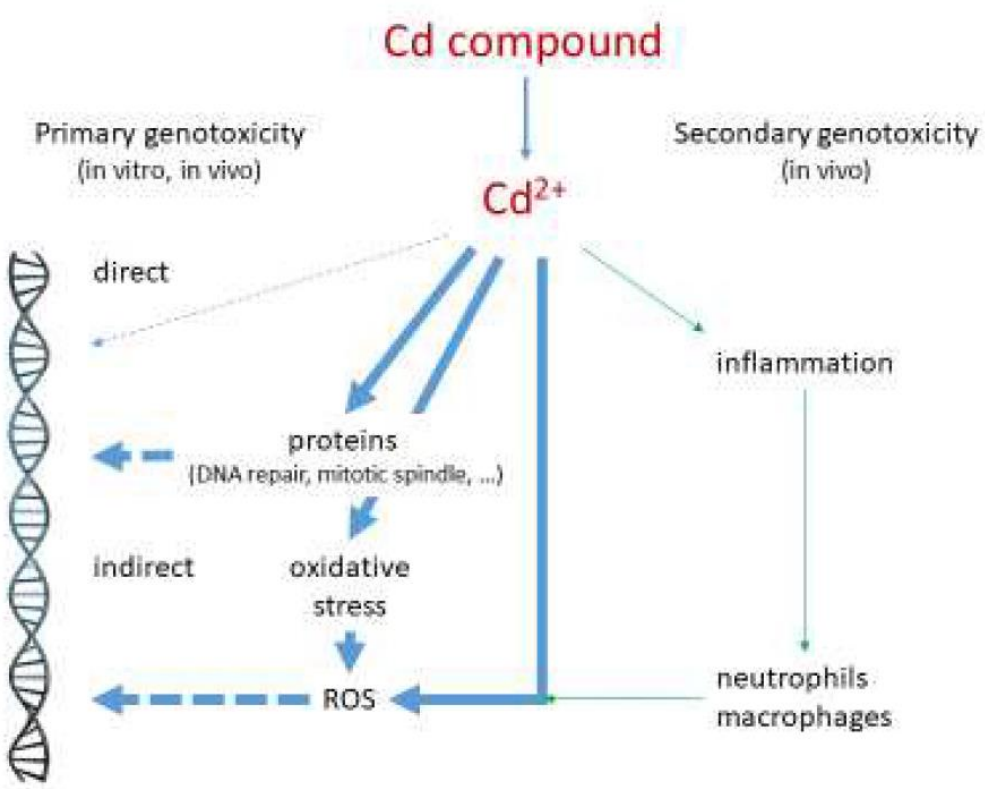

Figure: Mechanisms relevant for the genotoxic activity of Cd compounds (based on Hartwig et al. 2010)

To examine the possibility of documenting a threshold for the genotoxic effects of cadmium, the extensive *in vitro* database available for the soluble salt $CdCl_2$ was used as a reference. *In vitro* genotoxicity studies were reported graphically (Figures 1 to 6) (See in Annex) according to the analysed endpoints: induction of gene mutations (Fig 1), chromosomal aberrations (Fig 2), micronucleus (Fig 3), sister chromatid exchange (Fig 4) and DNA damage (Fig 5) as well as for all endpoints combined (Fig 6). Because of the diversity of experimental models (cell type, culture medium, …) and protocols for measuring and/or expressing genotoxic responses in the different

studies, it was not possible to merge the responses as expressed by the individual authors. Thus, in each figure, the responses were reported as a function of the tested concentrations with a value of 0 attributed to non-statistically significantly change compared to controls and a value of 1 attributed when statistically significant difference was observed compared to the controls, i.e. a statistically significant genotoxic response. Because of the large range of concentrations tested by the different authors, concentrations are reported on a logarithmic scale.

The data from experiments conducted by exposing cells to $CdCl_2$ in the absence of serum (SF: see table 1) were excluded as they yielded irrelevantly low effective doses (in part because of the acceleration of cellular cadmium uptake; Wang et al., 2001). Studies that analyzed protective effects of substances against cadmium-induced genotoxicity, and that reported data for only one concentration were also excluded, as well as studies that did not apply a statistical analysis. Concentrations inducing high levels of cytotoxicity (e.g. viability less than about 60%) were not included in the figures. When, in the same study, MN induction data were available for exposure with and without cytochalasin B, only data with cytochalasin B were included. When data on DNA damage and subsequent repair were presented, only DNA damage data were reported. Excluded studies and/or data are reported in italics in table 1.

The lowest concentrations inducing statistically significant genotoxic responses are reported in the following table for each endpoint as well as for all endpoints combined.

Endpoint	**Concentration**
Gene mutation	1 µM (Ochi and Oshsawa, 1983)
Chromosome aberration	1µM (Guerci et al., 2000)
Micronucleus	1 µM (Seoane and Dulout, 2001 ; Berces et al., 1993)
Sister chromatid exchange	1 µM (Mouron et al., 2004)
DNA damage	1 µM (Mouron et al., 2004) (0.01 µM Li et al., 2017a)
All endpoints combined	1 µM

Note: lowest concentrations inducing significant effects and corresponding references are reported. For the DNA damage endpoint, one study reported significant genotoxic effects at particularly low concentrations compared to all other studies (Li et al., 2017a). This study was considered as an outlier and excluded from the discussion.

It is interesting to observe the consistency of the lowest effective doses reported across all endpoints when studies using serum-free medium were excluded: 1 µM was the lowest effective dose for each considered endpoints and for all endpoints combined.

This finding is consistent with the existing knowledge on the mechanisms governing the genotoxic activity of $CdCl_2$ pointing towards the existence of thresholds.

3.3. Consistency of *in vivo* and human data with a threshold mechanism of genotoxicity

The available *in vitro* studies indicate that mechanisms governing the genotoxicity of cadmium (genotoxic mode of action) are threshold.

In vivo genotoxicity studies are reinforcing this evidence. The high doses required to elicit genotoxic effects *in vivo* (e.g. Mukherjee et al., 1988; Fahmy and Aly, 2000) are consistent with the presence of mechanism of action-based thresholds.

It is also important in this analysis of the evidence, to consider human epidemiology data. In the case of cadmium, the interpretation of epidemiological studies is most often complicated by simultaneous exposures to other genotoxic agents (e.g. arsenic in the cadmium smelters) and it is often unclear if the recorded effects are solely attributable to Cd exposure.

Of particular interest to supporting the existing knowledge on the mechanisms governing the genotoxic activity of Cd is the recent epidemiological study by Lison et al (2019) in manufacturing and recycling Ni-Cd batteries plants. This study is of particular interest as it presents several strengths compared to previous works, including an a priori power calculation, a careful selection of healthy workers covering a large array of Cd exposure levels documented by seniority, B-Cd and U-Cd, the use of the Cytochalasin-B MN-assay, the inclusion of positive controls, the documentation of an adjustment for potential confounders through multivariate statistical analyses. This study aimed at analyzing the shape of the dose-response relationship for the genotoxic effects of Cd in occupational settings. The authors did not record an increased frequency of MN, and therefore no dose-response relationship, in circulating lymphocytes of workers with a wide range of occupational exposure. These results are consistent with a threshold for systemic genotoxic effects of Cd, which is beyond the range of internal exposure levels measured (up to 12.5 µg/l B-Cd and 20 µg/l U-Cd).

Earlier epidemiological data suggest that chromosomal aberrations (CA), but not micronuclei (MN) appear in blood lymphocytes of workers in alloy (Cd, Zn, Ag, Cu) production plant with high Cd exposure, i.e. urinary Cd (CdU>10µg/L) (Forni, 1994; Forni et al., 1990), which is also supportive of a threshold. The CA levels did, however, not correlate with Cd-U, and this study is also limited in number of workers (n=40).

4. Discussion and conclusions: do we have evidence for a mechanism of action-based threshold?

The last SCOEL evaluation of cadmium and its inorganic compounds (2017) stated that "*Different and a priori non-mutually exclusive mechanisms for the carcinogenicity of Cd have been identified, including oxidative DNA damage, induction of oxidative stress (generation of reactive oxygen species), inhibition of DNA repair and deregulation of cell proliferation. All these mechanisms are non-stochastic and are characterized by a threshold below which no effect is expected. Cd is therefore considered by SCOEL as a Category C carcinogen, i.e. a genotoxic carcinogen for which a mode of action-based threshold can be identified, also called 'practical threshold' "*

The present review of the genotoxicity data for cadmium, which includes recent data published after the SCOEL review, shows that:

- *In vitro* mutation assays in bacterial test systems are negative.
- *In vitro, c*admium ions can induce gene mutations, cytogenetic alterations (CA, MN, SCE) and induction of DNA damage in mammalian cells.
- *In vivo* genotoxicity studies, confirm the *in vitro* findings, showing induction of gene mutations, DNA damage, induction of structural and numerical chromosome aberrations in somatic cells of mice, induction of micronuclei in somatic cells of mice and rats, and induction of numerical and structural chromosome aberrations in the germ cells of mice.
- Genotoxicity studies in exposed humans have produced mixed positive and negative responses but are often difficult to interpret due to co-exposure and other study limitations.

Thus, the current evidence indicates that exposure to cadmium compounds can induce genotoxicity and genomic instability through different molecular mechanisms. Cadmium does not induce direct DNA damage. In contrast, it induces increases in ROS formation, which in turn induce DNA damage and can also interfere with cell signaling. Other important mechanisms involved are interaction of cadmium with proteins involved in DNA repair, cell cycle checkpoints, apoptosis and interaction with epigenetic mechanisms. Cadmium can activate proto-oncogenes and inactivate tumor suppressor genes through interference with cell signaling and by epigenetic changes. All these features alone could contribute to genomic instability and probability of developing cancer or other diseases associated with genomic instability, but most likely their combination seems to be of particular importance (Hartwig et al. 2010, 2013).

Several lines of evidence in the present analysis of the genotoxicity data are pointing to non-stochastic mechanisms.

The genotoxicity of cadmium occurs through mechanisms that **indirectly** cause damage to DNA or chromosomes, via interactions with peptides and proteins. All those mechanisms are non-stochastic in nature, implying that a threshold exists below which no genotoxic effect is expected (SCOEL, 2017a).

The present analysis indicates that there is sufficient evidence that the dose-response relationships *in vitro* are compatible with a threshold. Lowest concentrations inducing statistically significant genotoxic effects could be identified, and consistency was shown across all genotoxicity endpoints.

The animal *in vivo* genotoxicity data reinforce this evidence for the genotoxic effects observed. Another element in the analysis of evidence is the recent study in healthy workers occupationally exposed to Cd (Lison et al., 2019) which is consistent with the existence of a threshold for genotoxic effects.

Overall, several lines of evidence are consistent with a mechanism of action-based threshold for the genotoxic activity of cadmium and cadmium compounds.

Several metals share similar mechanisms leading to genomic instability. For example, like cadmium, nickel can trigger similar genotoxic and epigenetic effects *in vitro*. Nickel compounds can also impair DNA repair, induce DNA damage, increase ROS production and induce epigenetic changes like increased methylation, increased histone phosphorylation and decreased histone acetylation. Similarly, thresholds for these *in vitro* genotoxic effects of nickel have been reported.

Another example is cobalt, where besides induction of oxidative damage (ROS), upregulation of hypoxia responsive genes, also inflammation is reported as an indirect mechanism for genotoxicity (NTP, 2014)

In summary, it can be concluded that based on the available lines of evidence, cadmium (Cd^{2+}) compounds should be considered as indirectly genotoxic with a mechanism-based threshold. Secondary genotoxicity resulting from local inflammation might also be considered, mainly upon inhalation exposure. This secondary genotoxic mechanism should also be characterized by a threshold dose-response relationship.

ANNEX

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Tables

Table 1: Summary table *in vitro* studies (CdCl2)

Endpoint	Species (test system)	Concentrations	Results	Remarks	Reference
	***In vitro* studies**				
Gene mutation	Salmonella typhimurium (Ames)	0.05, 0.5, 5, 50, 500 µg/plate	- (with and without S9 activation)		Bruce and Heddle (1979)
Gene mutation	Salmonella typhimurium (Ames)	0, 10, 33, 100, 333, 1000, 2000 µg/plate	-		Mortelmans et al. (1986)
Gene mutation	Chinese hamster lung fibroblasts (V79) (Hprt gene mutation Assay)	0, **1**, **2**, **3**, *4*, *5* and *6* µM	+	-Increase up to 3 µM; after 3 µM, effects disappear rapidly because of total inhibition (gross cytotoxicity) by metals. -marked inhibition of cell growth at 6 or 8 µM	Ochi and Ohsawa (1983)
Gene mutation	Chinese hamster lung fibroblasts (V79) (Hprt gene mutation Assay)	*0, 0.01,* ***0.1***, ***0.25***, *0.5, 1, 2,* ***4***, ***8***, *16 µM*	**+ (SF)**	Two peaks trend with significant increases at 0.1, 0.25 and 4, 8 µM	Jianhua et al. (2006)
Gene mutation	Chinese hamster lung fibroblasts (V79) (Hprt gene mutation Assay)	0, 2.5, **5**, **10**, *20, 30, 40 µ*M	+	-Concentration-dependent increase in mutation frequency plateaued at 20 µM -greatest toxicity observed between 10 and 40 µM (most cellular toxicity observed at 40 µM);	Gobrecht et al. (2017)
Gene mutation	Human lung fibroblast cell line (MRC-5) (Point mutation in codon 12 of K-ras protooncogene)	0, 1, 2 and 4 µM	-	Null or weak point mutation	Mouron et al. (2004)
Chromosomal aberration	Mouse spleen cells culture	0, 10, **15**, **20** µg/ml [0,54, **82**, **109** µM]	+	Concentration-dependent increase (significant at 15 and 20 µg/ml)	Fahmy and Aly (2000)

Chromosomal aberration	Chinese hamster skin, lung, kidney	*2 x 10^{-5} M [20 µM]*	+ (no stat)		Deaven and Campbell (1980)
Chromosomal aberration	Chinese hamster ovary cells (CHO)	*(i) 1 x 10^{-6} M [1 µM]* *(ii) 2 x 10^{-6} M [2 µM]*	+ (no stat)	(i) medium + 15 % newborn calf serum (ii) medium + 20% FCS	
Chromosomal aberration	Chinese hamster ovary cells (CHO-W8)	*0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M [0, 0.1, 1, 10, 100 µM]*	+ (no stat)	-increased % of cells with aberrations in each concentration (seems to be concentration-dependent -highest concentration (*10^{-4}* µM) proved toxic	Howard et al. (1991)
Chromosomal aberration	Chinese hamster ovary cells (CHO-W8)	(i)0, 0.4, ***0.8***, ***1.6***, ***3.2*** µM (ii)0, 1.6, 3.2, **6.3**, **12.5**, **25** µM (iii) 0, 0.3, 0.5, 1.0, 2.0 µM	+ (2h) (i) in the absence of 10% FCS **(SF)** (ii)in the presence of 10% FCS - (18h) (iii) in the presence of 10% FCS	the presence of 10% FCS during the 2h treatment period greatly retarded the cellular Cd intake and reduced CA induction	Wang and Lee (2001)
Chromosomal aberration	Human peripheral blood lymphocytes	0, 5 x 10^{-6} M and 5 x 10^{-5} [0, 5, 50 µM]	**- (SF)**		Deknudt and Deminatti (1978)
Chromosomal aberration	Human peripheral blood lymphocytes	*0, 10^{-4}, 10^{-3} and 5 x 10^{-3} mol/L [0, 100, 1000, 5000 µM]*	±	Not concentration dependent; statistical significance not reported for CA; interindividual variability; most frequent type: acentric fragments	Rozgaj et al. (2002)
Chromosomal aberration	Human peripheral blood lymphocytes	*0, **10^{-6}, 10^{-5}, 5 x 10^{-5}, 10^{-4}** mol/l [0, **1, 10, 50, 100** µM]*	**+ (SF)**	the higher concentrations [5 x10^{-5}, 10^{-4} mol/l] are cytotoxic (mitotic activity, apoptose)	Gateva et al. (2013)
Chromosomal aberration	Human lung fibroblast cell line (MRC-5)	0, **1, 2, 4 x 10^{-3}** mM [0, **1, 2, 4** µM]	+	Increased frequency of aneuploid cells at all tested concentrations	Güerci et al. (2000)
Micronuclei	Chinese hamster lung fibroblasts (V79)	0, **3**, **5** and ***10*** µM	+	-Concentration-dependent statistically significant increase in MN; MN predominantly CREST negative => clastogenic activity.	Ustundag et al. (2014)

				-cytotoxicity: IC80=6-12 μM, IC50= 4-8 μM	
Micronuclei	Chinese hamster lung fibroblasts (V79)	0, 2.5, 5, **10**, ***20*** and *30* μM (strong decrease in survival at 40 μM)	+	-Concentration-dependent increase up to 20 μM (significant at 10 and 20 μM), decreasing at 30 μM. -greatest toxicity observed between 10 and 40 μM (most cellular toxicity observed at 40 μM).	Gobrecht et al. (2017)
Micronuclei	Mouse lymphoma L5178Y cells	-(i): 0, **0.25, 0.5, 1**, *2, 4* μg/ml [0, **1.36, 2.73, 5.46,** *10.91, 21.82* μM] -(ii): 0, 0.21, **0.27, 0.34, 0.42, 0.52, 0.66** μg/ml [0, 1.15, **1.47, 1.86, 2.29, 2.84, 3.60** μM]	+	-(i) test 1: at concentrations giving 30% toxicity or less with and without cytokinesis-blocked method: significant increases in number of MN cells (relatively small at the lower concentrations tested without Cyto B); MN not scored at 2 and 4 μg/ml with and without Cyto B -(ii) test 2 to confirm results of test 1: at concentrations giving approximately 50% toxicity or less with and without cytokinesis-blocked method: significant increases in number of MN cells; at 0.21 μg/ml significant increase only without Cyto B	Fellows and O'Donovan (2010)
Micronuclei	Mouse lymphoma L5178Y cells	0, 0.23, 0.28, 0.33**, 0.40, 0.48, 0.58,** ***0.69*** μg/ml [0, 1.26, 1.53, 1.80, **2.18, 2.62, 3.16,** ***3.76*** μM]; 3h treatment	+	-statistically significant increase in number of MN cells at at least 28% cytotoxicity -50% cytotoxicity between 0.40 and 0.58 μg/ml	Lorge (2010)
Micronuclei	Chinese hamster ovary cells (CHO)	(i) 0, **0.26, 0.33, 0.41**, *0.51, 0.64, 0.80, 1.0* μg/ml [0, **1.42, 1.80, 2.24**, *2.78, 3.49, 4.36, 5.46* μM]	+	(i)with cytochalasin B in binucleate cells) (ii)without cytochalasin	Whitwell et al. (2010)

		(ii) *0, **0.26**, 0.33, 0.41, **0.51**, 0.64, **0.80, 1.0** µg/ml [0, **1.42**, 1.80, 2.24, **2.78**, 3.49, **4.36, 5.46** µM]*		B (in mononucleate cells)	
Micronuclei	Mouse cortical collecting duct cells (MCD4)	*0, **0.1** µM*	+	*significant decreased cell viability at 0.1 µM*	*Ranieri et al. (2019)*
Micronuclei	Human lung fibroblast cell line (MRC-5)	0, **1**, **2** and **4** µM	+	K_+ MN frequencies (arising from aneugenic events)= higher than K	Seoane and Dulout (2001)
Micronuclei	Human lymphoblastoid cells (TK6)	(i)0, 2, **4**, **6** and **8** µg/mL [0, 10.9, **21.8**, **32.7**, **43.6** µM] (ii) *0, 2, **4**, **6** and **8** µg/mL [0, 10.9, **21.8**, **32.7**, **43.6** µM]*	+	(i)with Cyto B in binucleate cells (ii)without Cyto B in mononucleate cells	Fowler et al. (2010)
Micronuclei	Human osteoblast-like MG-63 cells	0, **20,** ***50*** µM	+	significant inhibition of cell viability above 30 µM at 24 and 48h	Oliveira et al. (2014)
Micronuclei	Human hepatoblastoma cells (HepG2)	0, 0.25, **0.5, 1.0, 2.0** µM	**+ (SF)**	toxicity: IC50= 2.5 µM	Peng et al. (2015)
Micronuclei	Human peripheral blood lymphocytes	0, **10^{-6} , 10^{-5}, 10^{-4}, 10^{-3}** M for 30 min [0, **1, 10, 100, 1000** µM]	+		Berces et al. (1993)
Micronuclei	Human peripheral blood lymphocytes	0, **5**, **10** and ***35***µM	+	at 35 µM: massive occurrence of necrosis: more than 25% necrotic cells	Lewinska et al. (2008)
Micronuclei	Human peripheral blood lymphocytes	0, **3, 5** ppm [0, **16.4, 27.3** µM]	+		Turkez et al. (2012)
Micronuclei	Human peripheral blood lymphocytes	0, **10^{-6}** , **10^{-5}**, **10^{-4}** , *10^{-3}* M [0, **1**, **10**, **100,** *1000* µM]	± (ambiguous)	-interindividual variability. -cytotoxicity at 10^{-3} M	Kasuba and Rozgaj (2002)
Micronuclei	Human peripheral blood lymphocytes	0, 10^{-4}, 10^{-3} and 5 x 10^{-3} mol/L [0, 100, 1000, 5000 µM]	± (ambiguous)	Not concentration-dependent increase and interindividual variability	Rozgaj et al. (2002)
Micronuclei	Human peripheral blood lymphocytes	*0, 5 ppm [0, **27.3** µM]*	+ 1 dose		*Dirican and Turkez (2014)*

SCE	Mouse spleen cells	0, **10**, **15** and **20** μg/ml [0, **54**, **82**, **109** μM]	+	Concentration -dependent statistically significant increase	Fahmy and Aly (2000)
SCE	Chinese hamster ovary cells (CHO)	0, 4 x 10^{-7} M [0.4 μM]	-	marginal low toxicity at this dose	Deaven and Campbell (1980)
SCE	Chinese hamster ovary cells (CHO-W8)	*0, 10^{-8} , 10^{-7}, 10^{-6}, 10^{-5} M [0, 0.01, 0.1, 1, 10 μM]*	+ (no stat)	-Increased SCE (seems to be concentration-dependent) in each concentration -highest concentration (10^{-5} M) proved toxic	Howard et al. (1991)
SCE	Chinese hamster ovary cells (CHO-W8)	*(i)0, 0.25, 0.50, 1 and 2 μM* (ii)0, 0.3, 0.5, 1, 2 μM	- (2h) **(SF)** (i) in the absence of 10%FCS -(24h) (ii)in the presence of 10% FCS		Wang and Lee (2001)
SCE	Human lung fibroblast cell line (MRC-5)	0, **1**, **2** and **4** μM	+	-Not concentration-dependent -no cytotoxic effects demonstrated by the proliferation index	Mouron et al. (2004)
SCE	Human peripheral blood lymphocytes	0, **10^{-4}**, **10^{-3}** and **5 x 10^{-3}** mol/L [0, **100**, **1000**, **5000** μM]	+	Not concentration-dependent; interindividual variability	Rozgaj et al. (2002)
SCE	Human peripheral blood lymphocytes	0, **3, 5** ppm [0, **16.4, 27.3** μM]	+	SCE increase seems to be concentration-dependent	Turkez et al. (2012)
SCE	Human peripheral blood lymphocytes	*0, 5 ppm [0, **27.3** μM]*	+ 1 dose		*Dirican and Turkez (2014)*
SCE	Human peripheral blood lymphocytes	0, **50, 100, 200, 300, 400, 500,** *600* μM	+	-Concentration-dependent significant increase in SCE/cell -at 600μM peripheral blood lymphocytes failed to grow	Verma et al. (2019)
DNA damage	Chinese hamster lung fibroblasts (V79) (Comet assay)	*(i)0, 0.01, 0.1, **1**, **25**, **50**, **100** and **200** μM;* *(ii)Second assay: 0, 0.01, 0.1 and 1 μM with 0.5, 1, 2 and 4h as time of repair*	**+ (SF)**	(i)DNA damage: concentration dependent statistically significant increase in TL (significant from 1 μM) and in TD (significant from 25 μM) and % of comet cells (ii)DNA damage and repair: DNA damage decreased with	Jianhua et al. (2006)

				time of repair (close to normal level after 4h) but slower than with H_2O_2	
DNA damage	Chinese hamster lung fibroblasts (V79) (Comet assay)	0, **5** and **10** μM	+	Concentration-dependent significant increase of % tail intensity Cytotoxicity: IC80=6-12 μM, IC50= 4-8 μM	Ustundag et al. (2014)
DNA damage	Chinese hamster lung fibroblasts (V79) (Comet assay + histone H2AX phosphorylation)	0, **2.5**, **5**, **10**, ***20***, ***30*** and ***40*** μM	+	Concentration-dependent increase of SSB and DSB. SSB: significant increase of high TM up to 20 μM, and significant decrease at 30 and 40 μM probably due to large increase in cytotoxicity. DBS: detected at 10 μM and increased up to 40 μM.	Gobrecht et al. (2017)
DNA damage	Mouse embryonic fibroblast cell line (NIH3T3) (Comet assay)	0, 5, 10 μM	-	-No alterations of tail DNA % neither TM -Cd exposure at 5 or 10 μM did not induce changes in cell viability	Chen et al. (2016)
DNA damage	Mouse hippocampal cell line (HT-22) (Comet assay)	0, 2.8, 3.14, **3.26, 3.37** μM	+	-Concentration-dependent significant increase in % of DNA damage -cells treated in the range of IC10 to IC30	Karri et al. (2018)
DNA damage	Mouse cortical collecting duct cells (MCD4) (Comet assay)	*0, **1** μM*	+ 1 dose		*Ranieri et al. (2019)*
DNA damage	Chinese hamster ovary cells (CHO) (Alkaline sucrose gradient and DNA molecular weight calculations)	*0, 1, 10, 100 μM for 4, 4 and 3h, respectively*	+ (no stat)		Robison et al. (1982)

DNA damage	Chinese hamster lung fibroblasts (V79) (Alkaline elution)	*0, 2 x 10^{-5}, 5 x 10^{-5} and 2 x 10^{-4} M* *[0, 20, 50, 200 µM]*	± (no stat) (-without proteinase K digestion) (+ with proteinase K)	-without proteinase K digestion: no elution except at the higher concentration that was rather toxic -with proteinase K digestion: concentration-dependent increase in SSB	Ochi and Ohsawa (1983)
DNA damage	Mouse spermatocyte-derived cells (GC-2) (Oxidative DNA damage using expression level of γ-H2AX) (ROS measured with DCFH-DA)	*0, **20** µM*	+ 1 dose	-Cd induced DNA damage -tested times of treatment: 0, 0.5, 1, 2, 4, 6, 12 and 24h; significant increase of γ-H2AX after 12 and 24h treatment -Significantly increased levels of ROS	Li et al. (2017b)
DNA damage	Human lung fibroblast cell line (MRC-5) (Comet assay)	0, 1, 2, **4** µM	+	-Increased % of cells with tails at the higher concentration -in all cases cell viability was higher than 83%	Mouron et al. (2001)
DNA damage	Human lung fibroblast cell line (MRC-5) (Comet assay)	0, **1**, **2** and **4** µM	+	Significant not concentration dependent increase in CM as compared to control; slow (not significant) increment in TL and TM	Mouron et al. (2004)
DNA damage	Human liver carcinoma cells (HepG2) (Comet assay)	0, **1**, **2**, **3**, ***4*** and ***5*** µg/mL [0, **5.5**, **10.9**, **16.4**, ***21.8***, ***27.3*** µM]	+	Concentration-dependent increase of SSB: % of DNA damage (significant from 2 µg/mL), length of comet tail (significant from 1 µg/mL)	Skipper et al. (2016)
DNA damage	Human hepatoblastoma cells (HepG2) (Comet assay)	0, **10^{-8}, 10^{-7}, 10^{-6}** mol/l [0, **0.01, 0.1, 1** µM]	+	Significant increase in tail DNA %; seems to be concentration dependent -concentrations selected to ensure above 50% cell viability	Li et al. (2017a)
DNA damage	Human hepatoblastoma cells (HepG2) (Comet assay)	(i): 0, 5, **10, *50*** µM	+	-significant increases in DNA TI: (i): 1.21 and 1.25-fold cell viability: significant decrease at 50µM for 24h: (i)IC50= 13.96 µM	Lawal and Ellis (2010)

	Human astrocytoma cells (1321N1)	(ii): 0, 5, 10, ***50*** μM	+	(ii)IC50= 19.92 μM (ii): 1.44 fold	
	Human embryonic kidney cells (HEK293)	iii): 0, 5, 10, ***50*** μM	+	(iii)IC50= 44.8 μM (iii): 1.31 fold	
DNA damage	Human colon carcinoma cells (SW480) (Comet assay)	0, 2.5, **7.5, *15*** μg/ml [0, 22, **67, *134*** μM]	+	Significant increase of TI in the highest concentration; -significant increase of TL in the two higher concentrations; -cell viability decreased by 30% only at the highest concentration; increased ROS production only at the highest concentration	Curcic et al. (2014)
DNA damage	Human osteoblast-like cells (MG-63) (Comet assay)	(i)0, 20, 50 μM; 24h treatment (ii) 0, **20, 50** μM; 48h treatment	+	(i)no significant increases at 24h treatment (ii)significant increase in DNA fragmentation (% tail DNA) at both concentrations (higher at 20 μM) at 48h -cell viability: (i)IC30=68μM and IC50=91μM (ii)IC30=54μM and IC50=91μM	Oliveira et al. (2014)
DNA damage	Human dermal fibroblast cells (Comet assay)	0, 5×10^{-5}, **1×10^{-4}, 2×10^{-4}** and **5×10^{-4}** mol/L [0, 50, **100, 200, 500** μM]	+	-Significant increase of DNA strand breaks starting from 100 μmol/L -IC50 equal to 1×10^{-3}M	Belliardo et al. (2018)
DNA damage	Human Jurkat T cell line (Comet assay)	0, **5, 25, *50*** μM; 24h	+	Concentration-dependent significant increase in DNA damage -LC50(24h)= 40 μM	Nemmiche et al. (2011)
DNA damage	Human peripheral blood lymphocytes (Comet assay)	0, 10^{-4}, 10^{-3} and 5×10^{-3} mol/L [0, 100, 1000, 5000 μM]	± Ambiguous	Notable interindividual differences: significant increase in DNA migration was seen for one subject at all tested concentrations, only in the lower concentration in another subject and no significant differences	Rozgaj et al. (2002)

				between treated and control samples in the two last subjects	
DNA damage	Human peripheral blood cells (Comet assay)	0, **50, 100, 200, 300, 400, 500** μM	+	Concentration-dependent increase in TM -at 600μM peripheral blood lymphocytes failed to grow	Verma et al. (2019)
DNA damage	Human epithelial larynx tumor cell line (SQ20B) (Comet assay + histone H2AX phosphorylation)	0, **25** and **50** μM (48h)	+	Concentration-dependent increase of DNA damage: TI (DNA%), TL and incidence of AST and DSB -cytotoxicity 48h: IC50=61 μM , IC30=50 μM , IC10=25 μM	Trabelsi et al. (2016)
DNA damage	Human hepatoblastoma cells (HepG2) (histone H2AX phosphorylation)	*(i): 0, 1, 10,* ***25, 50****, 75, 100 μM; 24h treatment*	**+ (SF)**	-concentration dependent increase in γ H2AX (more than 1.5-fold increase at 50 μM); cytotoxicity observed from 25 μM (LOAEC), very high at 75 and 100 μM	Kopp et al. (2018)
	Human epithelial colorectal adenocarcinoma cells (LS-174T)	*(ii):0, 1, 10, 25,* ***50, 75****, 100 μM; 24h treatment*	**+ (SF)**	-concentration dependent increase in γ H2AX at subtoxic concentrations; LOAEC= 50 μM	
DNA damage	Human adenocarcinomic alveolar basal epithelial cells (A549) (alkaline unwinding technique in combination with bacterial formamidopyridine-DNA-glycosylase (Fpg))	0, 10, 25, 50, ***75*** μM	+	Significant induction of DNA strand breaks and Fpg-sensitive sites only at 75 μm which was strongly cytotoxic	Schwerdtle et al. (2010)

Abbreviations: -, negative results; +, positive results; ±, weakly positive results, interindividual variability; SF, experiments conducted by exposing cells in the absence of serum. Hprt, hypoxanthine guanine phosphoribosyl transferase; cyto B, cytochalasin B; CREST, anti-centromere antibodies ; $IC_{10, 30, 50, 80}$, concentration which inhibited 10%, 30%, 50%, 80% of growth, DCFH-DA: ROS-detecting fluorescent dye; γ -H2AX, a marker of DNA damage, AST, abnormal size tails; CM, comet moment; SSB, single strand breaks; DSB, double strand breaks; K_+, kinetochore positive; K_-, kinetockore negative

Note: concentrations are in bold when statistically significant differences with controls were observed; concentrations not included in the dose-response analysis (Section 2.2.2) are in italics i.e. when studies examined protective effects of substances against Cd-induced genotoxicity, and reported data for only one Cd concentration, data from experiments conducted by exposing cells in the absence of serum, studies that did not apply a statistical analysis, concentration inducing high levels of cytotoxicity [e.g. viability lower than about 60%], MN induction data for treatment without Cyto B when data for both treatments [with and without CytoB] are presented and DNA repair data when DNA damage and repair are presented.

Table 2: Summary table *in vivo* animal studies (CdCl2)

Endpoint	Species (test system)	Concentrations	Results	Reference
	***In vivo* studies**			
	Somatic cells			
Gene mutation	Rat lymphocytes (males)	0, 1, **5**, **10** mg/kg bw; single injection	+ (ip)	Jianhua et al. (2006)
Chromosomal aberration	Mouse bone marrow (males)	0, **20**, **40**, **60** µmol/kg bw/injection; 1 injection/day; 3 days) [0, **3.67**, **7.33**, **11** mg/kg bw]	+ (sc)	El-Habit and Moneim (2014)
Chromosomal aberration	Mouse bone marrow (males)	0, **0.42**, **0.84**, **1.68**, **3.37**, **6.75** mg/kg bw; single injection	+ (ip)	Mukherjee et al. (1988)
Chromosomal aberration	Mouse bone marrow (males)	0, 1.9, **5.7**, **9.5** mg/kg bw; single injection	+ (ip)	Fahmy and Aly (2000)
Micronuclei	Rat peripheral blood erythrocytes (i) + bone marrow (males) (ii)	0, **15** mg/kg bw/d; (i)for 60 days (ii)for 24h	+ (o) 1 dose	Celik et al. (2009)
Micronuclei	Rat peripheral blood lymphocytes (suckling rats)	-orally: 0, **0.5** mg/kg bw/day; 9 days; total: 4.5 mg/kg bw -subcutaneously: 0, **0.5** mg/kg bw; single injection	+ (o, sc) 1 dose	Kasuba et al. (2002)
Micronuclei	Mouse bone marrow cells (males)	0, **20**, **40**, **60** µmol/kg bw/injection; 1 per day; 3 days [0, **3.67**, **7.33**, **11** mg/kg bw]	+ (sc)	El-Habit and Moneim (2014)
Micronuclei	Mouse bone marrow reticulocytes (females)	~ 0, 1.875, 3.75, 7.5, 15 mg/kg; 1 injection/day; 5 days	– (ip)	Bruce and Heddle (1979)
Micronuclei	Mouse polychromatic erythrocytes (males)	0, 0.42, 0.84, 1.68, 3.37, **6.75** mg/kg bw; single injection	+ (ip)	Mukherjee et al. (1988)
Micronuclei	Mouse bone marrow erythrocytes (males)	0, 0.025, **0.050**, **0.1**, **0.25**, **0.5**, **1**, **2** mg/kg bw; single injection	+ (ip)	Jagetia and Adiga (1994)

Micronuclei	Mouse bone marrow erythrocytes (males, females)	0, **1.9**, **5.7**, **7.6** mg/kg bw; single injection	+ (ip)	Fahmy and Aly (2000)
Micronuclei	Mouse bone-marrow erythrocytes (males, females)	0, **7, 8, 9, 10 and 11** mg/kg bw; post-treatment time: 24h and 48h	+ (ip)	Viswanadh et al. (2010)
SCE	Mouse bone marrow (males)	0, 0.42, **0.84**, **1.68**, **3.37**, **6.75** mg/kg bw; single injection	+ (ip)	Mukherjee et al. (1988)
SCE	Mouse bone marrow (males)	0, 1.9, **5.7**, **7.6** mg/kg bw; single injection	+ (ip)	Fahmy and Aly (2000)
DNA damage	Mouse brain, bone marrow, nasal epithelial cells, lung, leukocytes, testicle, liver, kidney (males) (comet assay)	0, **0.08** μg/cc; -1 single inhalation; -3 inhalations (2wks) -5 inhalations (3wks) -7 inhalations (4wks)	+ (inh) 1 dose	Valverde et al. (2000)
DNA damage	Mouse leucocytes (males) (comet assay)	0, 0.5, **1**, **2**, **4, 8, 16, 32, 64, 128** mg/kg bw; single administration	+ (o)	Devi et al. (2001)
DNA damage	Mouse (immature) lung (comet assay)	0, 1.87, **3.74, 7.48** mg/kg bw; for 40 days	+ (o)	Yang et al. (2012)
DNA damage	Mouse gastrointestinal tract (duodenum + colon) (females) (i)classic comet assay (FpG-) (ii)modified comet assay (FpG+) to detect oxidative DNA damage	(i)0, **5**, 20, 100 ppm (=mg/l); for 8 weeks (ii)duodenum: 0, 5, 20, 100 ppm; for 8 weeks; colon: 0, **5, 20, 100** ppm for 8 weeks	+ (o)	Breton et al. (2013)
DNA damage	Mouse blood (males) (comet assay)	0, 0.1, 0.5, **1, 2** mg/L; for 30 days treatment continuously	+ (o)	Agnihotri et al. (2015)
DNA damage	Mouse liver (i), stomach (ii) (males)	(i)0, **20, 40 and 80** mg/kg/d; for 3 days; (ii) 0, 20, **40 and 80 m**g/kg/d; for 3 days	+ (o)	Wada et al. (2015)

	(comet assay)		(considered as equivocal by the authors)	
DNA damage	Rat cardiomyocytes (males) (comet assay)	0, **5** mg/kg bw/day; for 30 days	+ (o) 1 dose	Ghosh and Indra (2018)
DNA damage	Rat reticulocytes (suckling rats) (comet assay)	-orally:0, **0.5** mg/kg bw/day; 9 days; total: 4.5 mg/kg bw -subcutaneously: 0, **0.5** mg/kg bw; single injection	+ (o, sc) 1 dose	Kasuba et al. (2002)
DNA damage	Rat kidney, lung (males) Alkaline unwinding assay followed by phenol single-strand extraction of DNA (lung, liver and kidney)	0, **4** mg/kg bw; single injection	+ (ip) 1 dose	Saplakoglu et al. (1997)
	Rat liver	0, **4** mg/kg bw; single injection	-	
	Germ cells			
Dominant lethal mutations	Rat spermatogenesis	0, 0.1, 1, 10 mg/kg bw/day; 12-15 wks	– (o)	Sutou et al. (1980)
Dominant lethal mutations	Mouse spermatogenesis	0, 5.4, 7.0, 1.35, 2.70 mg/kg; single injection	– (ip)	Epstein et al. (1972)
Dominant lethal mutations	Mouse spermatogenesis	0, 0.5, 1.75, 3 mg/kg bw; single injection	– (ip)	Gilliavod and Leonard (1975)
Dominant lethal mutations	Mouse oocytes	0, 2 mg/kg bw; single injection	– (ip) 1 dose	Suter (1975)
Chromosomal aberration	Hamster oocytes	0, 1, **2**, **4** mg/kg bw; single injection	+ (sc)	Watanabe et al. (1979)
Chromosomal aberration	Mouse oocytes	0, **1.5**, **3** mg/kg bw; single injection	+ (sc)	Watanabe et al. (1982)
Chromosomal aberration	Mouse oocytes	0, 2, 4, 6 mg/kg bw; single injection	– (ip)	Mailhes et al. (1988)
Chromosomal aberration	Mouse spermatocytes	0, **1**, **3**, **6** mg/kg; single injection	+ (ip)	Miller and Adler (1992)

Chromosomal aberration	Mouse spermatocytes	0, 0.9, 1.9, **5.7** or 9.5 mg/kg bw; single injection	+ (ip)	Fahmy and Aly (2000)
Chromosomal translocations	Mouse spermatocytes	0, 0.5, 1.75, 3 mg/kg bw; single injection	– (ip)	Gilliavod and Leonard (1975)
DNA damage	Rat spermatocytes (Comet assay)	0, **25, 50** mg/L; 13 weeks, 90 days	+ (o)	Nava-Hernandez et al. (2009)
Oxidative DNA damage	Mouse spermatocytes (Oxidative *in vivo* damage in testis – 8-OH-dG level measured by ELISA test)	0, **2** mg/kg bw/d for 7 days	+ (ip) 1 dose	Li et al. (2017b)

Abbreviations: -, negative results ; +, positive results ; ±, weakly positive results; inh, administration by inhalation; ip, intraperitoneal injection; o, oral administration; sc, subcutaneous injection.

Note: doses are in bold when statistically significant differences with controls were observed

Table 3: Summary table-Human studies

Species (test system)	Endpoint	Results	Reference
Occupational exposure			
Inhalation – workers			
Human peripheral blood lymphocytes	Chromosomal aberration	± / ND	Deknudt et al., 1973
Human peripheral blood leukocytes	Chromosomal aberration	± / ND	Deknudt and Leonard, 1975
Human peripheral blood lymphocytes	Chromosomal aberration	-/ND	Bui et al., 1975
Human peripheral blood lymphocytes	Chromosomal aberration	+ / -	Bauchinger et al., 1976
Human peripheral blood lymphocytes	Chromosomal aberration	-/ -	O'Riordan et al., 1978
Human peripheral blood lymphocytes	Chromosomal aberration	-/ND	Fleig et al., 1983
Human peripheral blood leukocytes	Chromosomal aberration	+ (high intensity, long-term exposure) / -	Forni et al., 1990; Forni 1994
Human peripheral blood leukocytes	Chromosomal aberration	+ / -	Abrahim et al., 2011
Human peripheral blood lymphocytes	Micronuclei	-/ ND	Forni 1994
Human peripheral blood lymphocytes	Micronuclei	+ / ND	Hamurcu et al., 2001
Human peripheral blood lymphocytes	Micronuclei	+/-	Palus et al., 2003
Human peripheral blood lymphocytes	Micronuclei	-/ -	Wegner et al., 2004
Human peripheral blood lymphocytes	Micronuclei	+ / -	Iarmacovai et al., 2005
Human peripheral blood lymphocytes	Micronuclei	+ / -	Kasuba et al., 2010
Human peripheral blood lymphocytes	Micronuclei	+ / +	Kasuba et al., 2012
Human peripheral blood lymphocytes	Micronuclei	-/ -	Lison et al., 2019
Human peripheral blood lymphocytes	Sister chromatid exchange	+ / +	Abrahim et al., 2011
Human peripheral blood lymphocytes	Sister chromatid exchange	+ / -	Palus et al., 2003
Human peripheral blood lymphocytes	Sister chromatid exchange	+ / +	Wegner et al., 2004
Mononuclear blood cells	DNA damage	ND / +	Hengstler et al., 2003
peripheral blood lymphocytes	DNA damage	+ / -	Palus et al., 2003
Human peripheral blood lymphocytes	DNA damage	+ / -	Iarmarcovai et al., 2005
Human peripheral blood lymphocytes	DNA damage	+ / -	Botta et al., 2006
Human peripheral blood lymphocytes	DNA damage	+ / +	Kasuda et al., 2012
Human lung cells (sputum cells)	DNA damage	+ / +	Moitra et al., 2015
Human peripheral blood lymphocytes	DNA damage	+ / +	Sani and Abdullali, 2016
Human peripheral blood leukocytes	DNA damage	+ / +	Rashid et al., 2018

Results are presented as comparison between exposed and control group / correlation or regression results; +, positive results; -, negative results; ND, data not available.

Figures

figures from Van Maele-Fabry G and Lison D (2020)

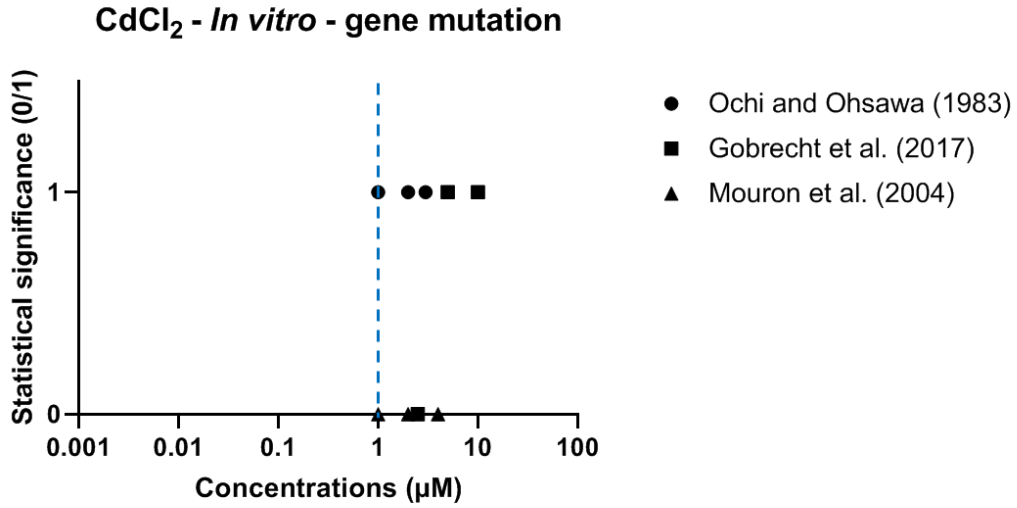

Figure 1. Dose-response relationship for gene mutations induced by cadmium chloride.
For each dose tested, the response was coded "1" or "0" when a statistically significant effect was reported or not, respectively.
The dashed line represents the lowest concentration inducing a genotoxic effect *in vitro*.
Data not included in the figure were:
- from experiments conducted by exposing cells in the absence of serum,
- concentrations inducing high levels of cytotoxicity [e.g. viability lower than about 60%].

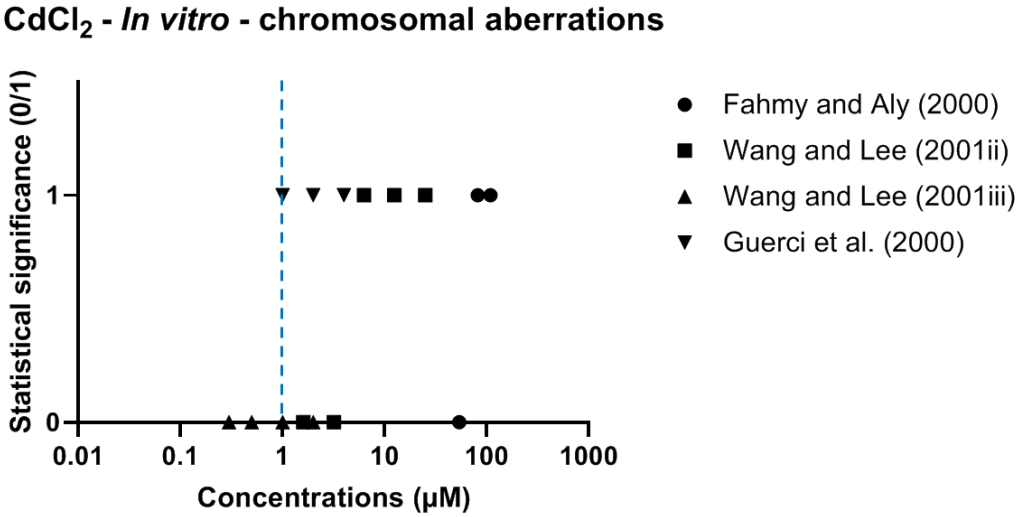

Figure 2. Dose-response relationship for chromosome aberrations induced by cadmium chloride
For each dose tested, the response was coded "1" or "0" when a statistically significant effect was reported or not, respectively.
The dashed line represents the lowest concentration inducing a genotoxic effect *in vitro*.
Data not included in the figure were:
- from experiments conducted by exposing cells in the absence of serum,
- concentrations inducing high levels of cytotoxicity [e.g. viability lower than about 60%],
- from studies that did not apply a statistical analysis.

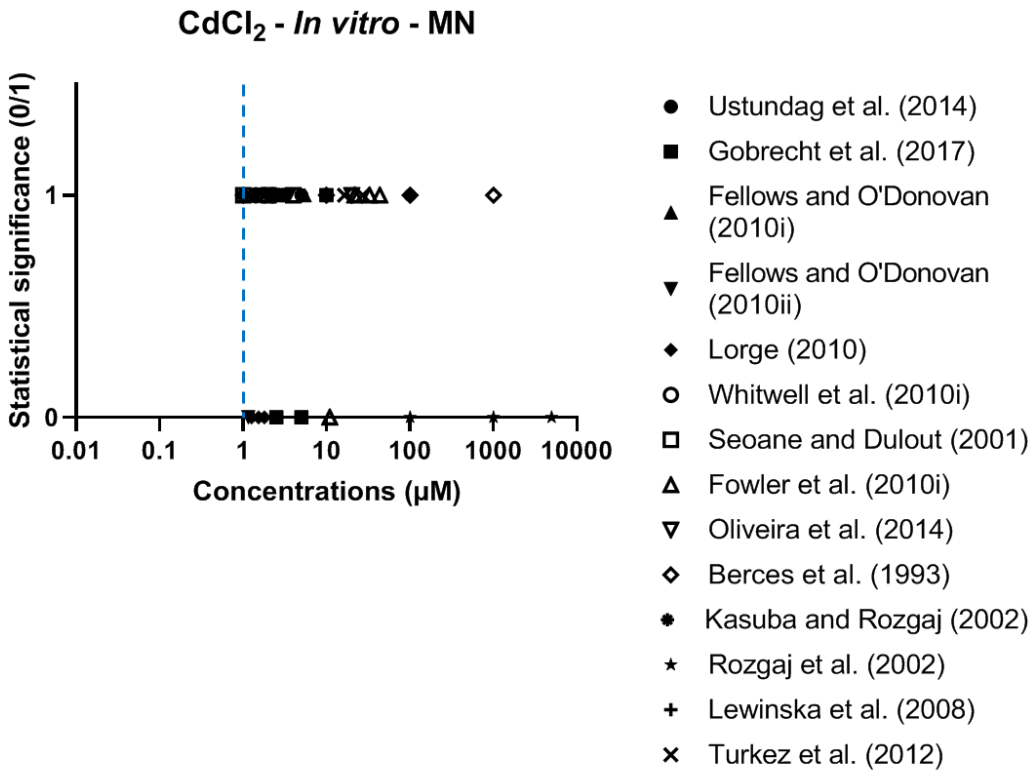

Figure 3. Dose-response relationship for micronuclei induced by cadmium chloride

For each dose tested, the response was coded "1" or "0" when a statistically significant effect was reported or not, respectively.

The dashed line represents the lowest concentration inducing a genotoxic effect *in vitro*.

Data not included in the figure were:

- from experiments conducted by exposing cells in the absence of serum,
- concentrations inducing high levels of cytotoxicity [e.g. viability lower than about 60%],
- from studies that examined protective effects of substances against Cd-induced genotoxicity, and reported data for only one Cd concentration,
- MN induction data for treatment without Cyto B when data for both treatments [with and without CytoB] are presented.

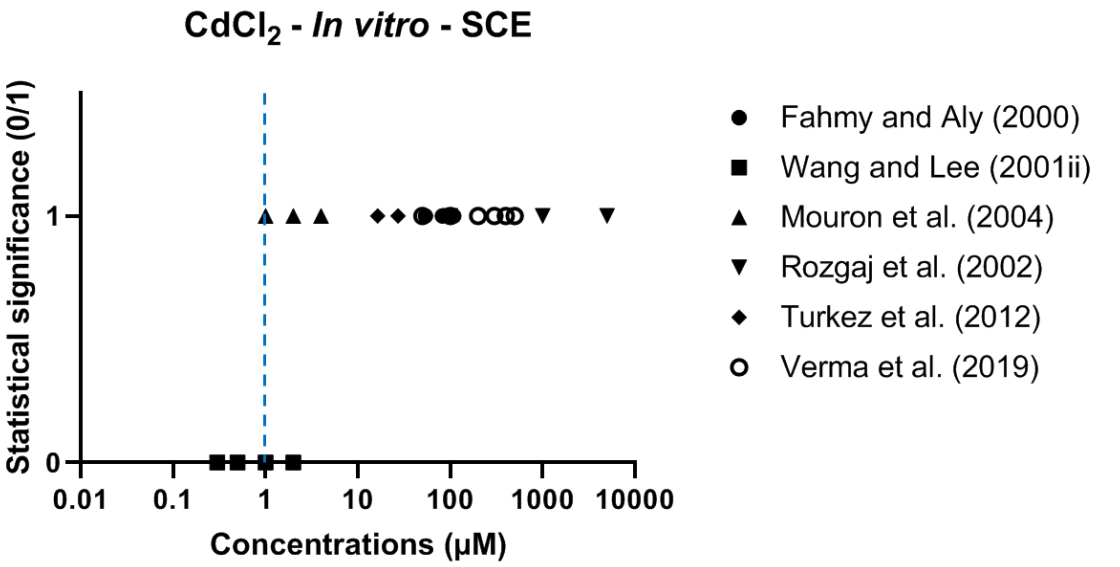

Figure 4. Dose-response relationship for sister chromatid exchanges induced by cadmium chloride

For each dose tested, the response was coded "1" or "0" when a statistically significant effect was reported or not, respectively.

The dashed line represents the lowest concentration inducing a genotoxic effect *in vitro*.

Data not included in the figure were:

- from experiments conducted by exposing cells in the absence of serum,
- concentrations inducing high levels of cytotoxicity [e.g. viability lower than about 60%],
- from studies that did not apply a statistical analysis,
- from studies that examined protective effects of substances against Cd-induced genotoxicity, and reported data for only one Cd concentration.

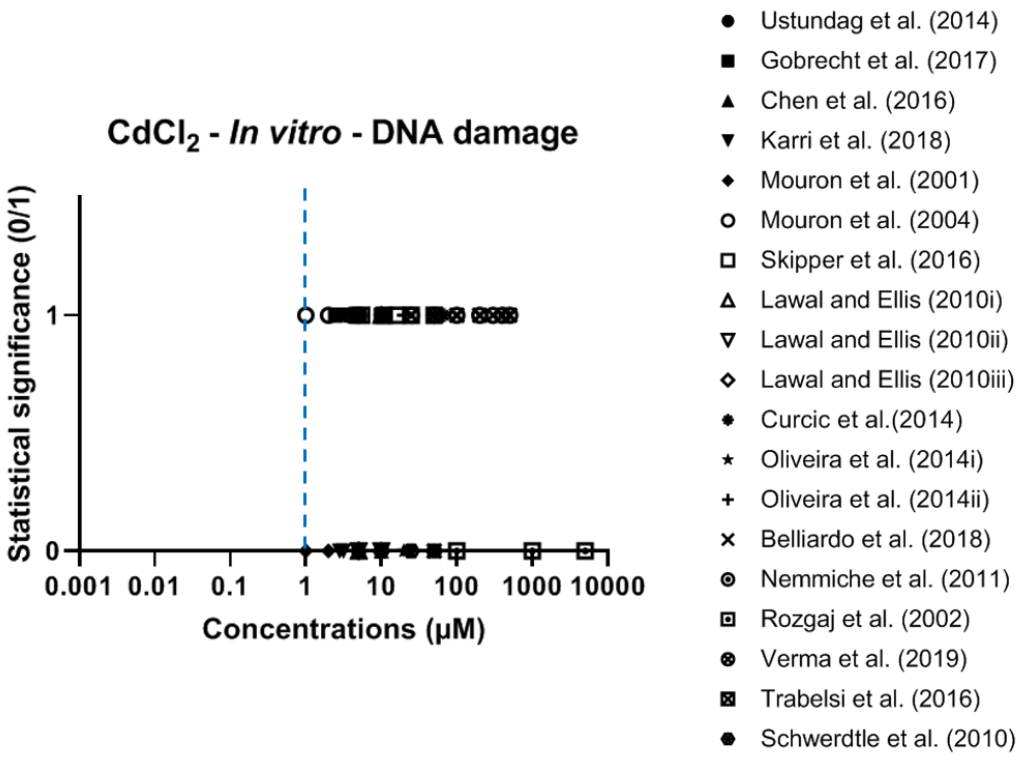

Figure 5. Dose-response relationship for DNA damages induced by cadmium chloride

For each dose tested, the response was coded "1" or "0" when a statistically significant effect was reported or not, respectively.

The dashed line represents the lowest concentration inducing a genotoxic effect *in vitro*.

Data not included in the figure were:

- from experiments conducted by exposing cells in the absence of serum,
- concentrations inducing high levels of cytotoxicity [e.g. viability lower than about 60%],
- from studies that examined protective effects of substances against Cd-induced genotoxicity, and reported data for only one Cd concentration,
- from studies that did not apply a statistical analysis,
- DNA repair data when DNA damage and repair are presented.

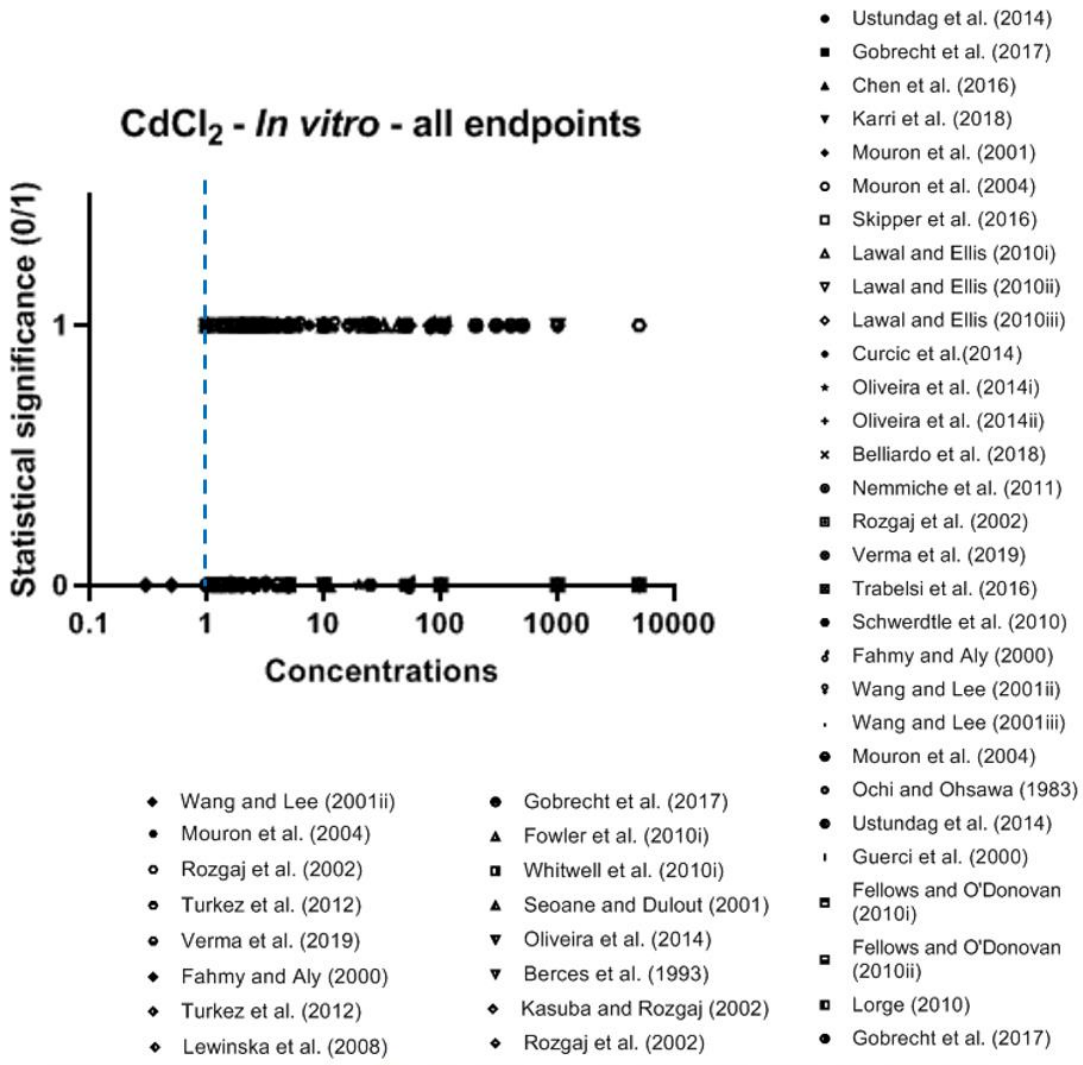

Figure 6. Dose-response relationship for all genotoxic effects induced by cadmium chloride

For each dose tested, the response was coded "1" or "0" when a statistically significant effect was reported or not, respectively.

The dashed line represents the lowest concentration inducing a genotoxic effect *in vitro*.

Data not included in the figure were:

- from experiments conducted by exposing cells in the absence of serum,
- concentrations inducing high levels of cytotoxicity [e.g. viability lower than about 60%],
- from studies that examined protective effects of substances against Cd-induced genotoxicity, and reported data for only one Cd concentration,
- from studies that did not apply a statistical analysis,
- MN induction data for treatment without Cyto B when data for both treatments [with and without CytoB] are presented,
- DNA repair data when DNA damage and repair are presented.