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Mini review

Review of the genotoxicity of "Arvin compounds", drinking water contaminants formed by the degradation of antoxidants in polyolefin pipes

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ABSTRACT

Polyolefin pipes used in drinking water distribution systems require a number of functional additives to ensure stability and durability. Some of these additives and/or their degradation products may migrate from the pipes into the drinking water. Previously, a number of branched chain alkylphenol degradants have been identified in drinking water; these were termed "Arvin substances" and numbered Arvin 1–10. As potential genotoxicity is a human health safety concern, the genotoxicity of Arvin substances is reviewed based on comprehensive *in vitro* and *in vivo* data available. Results obtained from genotoxicity studies addressing mutagenicity and clastogenicity are available for nine of the ten Arvin substances. These nine Arvin substances were consistently negative in bacterial mutagenicity studies. Divergent results were obtained in clastogenicity assays with some positive responses induced by the branched chain alkylphenols Arvin 1, 2, and 4, often accompanied by significant cytotoxicity. However, Arvin 1, 2, and 4 did not induce micronuclei or genotoxicity *in vivo* during follow-up testing. The other Arvin compounds did not show genotoxic activity *in vitro*. In conclusion, regarding human health risk characterization, the Arvin compounds are not considered genotoxic agents based on the available data.

1. Introduction

Polyolefin pipes are widely used for drinking water transport from processing sites to end users due to their durability, favorable mechanical properties, and easy handling during installation. To obtain highly stable polyolefins (such as polyethylene) that permit pipes being applicable for periods of up to 100 years, antioxidants and other stabilizers have to be added. Antioxidants contained in polyolefins may form reaction/degradation products during high temperature processing. Of note, the formation of transformation products from antioxidants in polyethylene is strongly dependent on storage, handling, and processing conditions (Dopico-García et al. 2007; Hahladakis et al. 2018). These reaction/degradation products may migrate from the polyethylene pipes into drinking water, resulting in human exposure (Löschner et al. 2011; Lützhøft et al. 2013). A number of organic compounds migrating from polyethylene pipes into drinking water were first identified more than 20 years ago (Brocca et al. 2002) and are often termed "Arvin substances" (Löschner et al. 2011). These represent a series of degradants of branched chain alkyl phenols (structures in Fig. 1) and are numbered Arvin 1 to Arvin 10.

The formation of Arvin 3, 4, 6, 7, 8, 9, and 10 can be readily explained by hydrolytic degradation of the polyolefin stabilizers pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate, tris(2,4-di-*tert*-butylphenyl)phosphite, and octadecyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate (Löschner et al. 2011). In contrast, as the basic structures of Arvin 1 (4-ethylphenol) and Arvin 2 (*p-tert*-butylphenol) are not present in a hydrolysable form in these stabilizers, their presence may be due to impurities in some of the polyolefin stabilizers or due to other sources, e.g. natural sources as shown for 4-ethylphenol (Api et al. 2021). Arvin 5 was not detected in migration studies with drinking water pipes, it is therefore considered an artefact.

Since Arvin substances are rather lipophilic, their migration from polyethylene pipes into drinking water is limited and they are usually found in drinking water in concentrations in the low μg/L range (Löschner et al. 2011; Ryssel et al. 2015). Thus, human exposure to these degradation products via drinking water is estimated to be in the range of up to a few μg/kg bw/day. Considering the low exposure levels, the toxicological endpoint of concern is the ability of these compounds to interfere with genetic information (DNA and chromosomal structures) and induce DNA- and chromosomal-damage (genotoxicity) potentially

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as an initiating event for the formation of tumors (Bolt, 2003; Nohmi, 2018).

As thresholds may be absent for genotoxicity and a potential tumor risk may be present due to genotoxic agents even at low doses, the ability of a chemical to induce genotoxicity is an integral part of toxicity testing of chemicals and a basic prerequisite for drinking water contact materials. Genotoxicity testing is an important aspect in the prediction of potential carcinogenicity and relies on a battery of well-established *in vitro* tests, both in bacteria and mammalian cells (EFSA 2011a; Nesslany, 2017). The protocols used apply *in vitro* assays which evaluate either the potential of a chemical to induce gene mutations or the ability of a substance to induce chromosomal damage or cytogenicity (SCCS, 2012). Assessment of genotoxicity endpoints in intact animals is only performed if inconsistent results are obtained in the *in vitro* tests employed or if further evaluation due to positive *in vitro* results is required. The methods of choice for *in vivo* testing are the micronucleus test for concerns regarding clastogenicity and aneugenicity, the transgenic rodent assay (TGR) or pig-a assay for mutagenicity, and the Comet assay as general indicator test for genotoxicity. All of the described *in vitro* and *in vivo* test procedures are standardized and covered by OECD testing guidelines.

The scope of this publication is to review the available genotoxicity information on Arvin substances and address a potential for genotoxicity due to the possible migration of these compounds from polyolefin pipes into drinking water and ensuing human exposures.

2. Approach

The information on genotoxicity of the Arvin substances was obtained from original study reports available for most of these substances. Most studies were performed following OECD guidelines for testing of chemicals and under "good laboratory practice" and included the required number of concentrations and repeat experiments. The study reports were provided by manufacturers of polyolefin stabilizers and their trade organization. Some information was also retrieved from registration dossiers on the website of the European Chemicals Agency (ECHA) using the respective CAS registry number for search. To be able to include studies not provided by the manufacturers, information on effects of Arvin compounds were also searched in Pubmed using the respective CAS registry number and the search terms "mutagenicity", "genotoxicity", and "micronucleus induction". This search did not retrieve additional studies in the public domain.

3. Review of available genotoxicity studies

Results from *in vitro* studies investigating mutagenicity and chromosome damage/cytogenicity in mammalian cells are available for nine out of the ten Arvin substances. *In vivo* short-term studies such as the mouse bone marrow micronucleus test were only performed when the *in vitro* studies gave inconsistent or positive results and thus results from *in vivo* genotoxicity testing tests are only available for three Arvin substances (see Table 1).

3.1. 4-Ethylphenol (CAS 123–07-9), „Arvin 1"

4-Ethylphenol is included in the list of Arvin substances but has not been consistently reported in studies on the migration of chemicals from polyethylene (Brocca et al. 2002; Diera et al. 2023; Löschner et al. 2011). Studies on the genotoxicity of 4-ethylphenol were commissioned by the Japanese government in their "Existing chemical substances safety evaluation". The evaluation in this review is based on translations of the original reports and the original reports on the *in vivo* micronucleus assay. Study results on genotoxicity endpoints for 4-ethylphenol are also available in the REACH registration dossier for 4-ethylphenol (ECHA, 2022) and are summarized in a published review article (Api et al. 2021).

3.1.1. Bacterial mutagenicity assay

The mutagenicity of 4-ethylphenol was assessed in a bacterial reverse mutation assay following OECD Test guideline (TG) 471 in the recommended strains of *Salmonella* (*S.*) *typhimurium* (TA100, TA1535, TA98, and TA1537) and in *Escherichia (E.) coli* WP2 *uvrA* in the presence and absence of a metabolic activation system (S9, obtained from rat liver) (FDSC 2000a). The study included positive controls as recommended in the OECD TG and six concentrations of 4-ethylphenol in dimethyl sulfide (DMSO) (62.5, 125, 250, 500, 1000 and 2000 μg/plate) were applied. The experiments were conducted in triplicates by the preincubation method and were repeated for confirmation. Applicable concentrations were limited by bacterial toxicity observed at ≥ 1000 μg/plate in the absence of S9 and at 2000 μg/plate in the presence

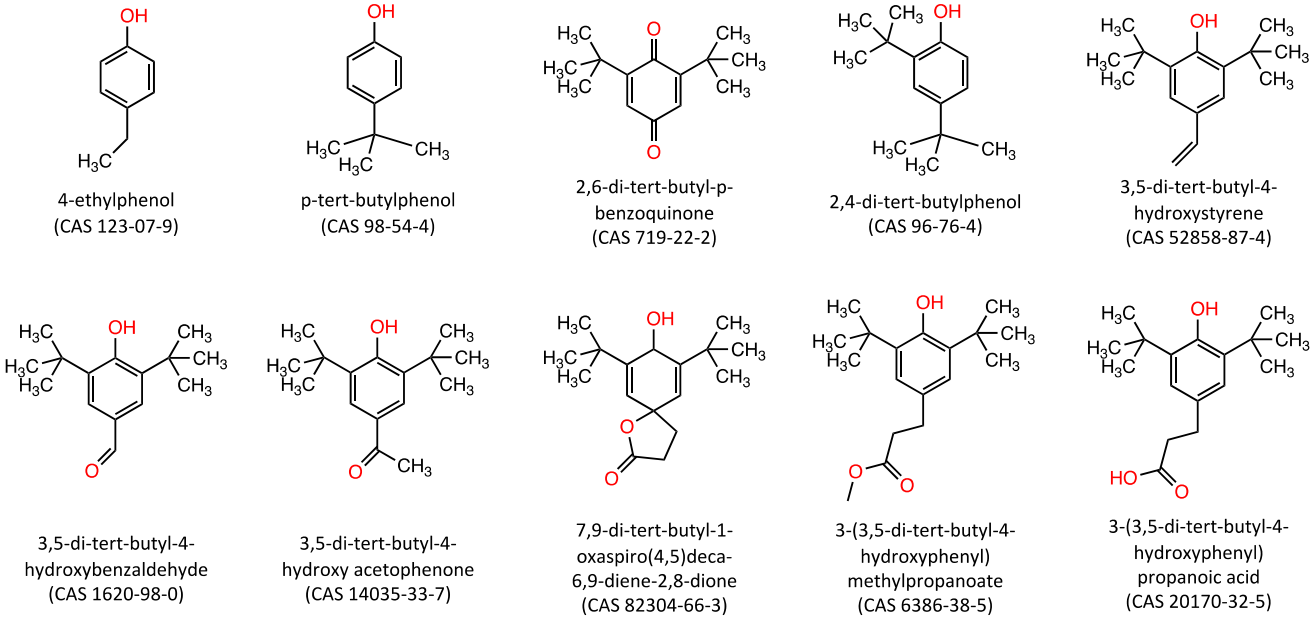

Fig. 1. Chemical structures of "Arvin compounds" that may migrate from thermally processed polyolefins as degradation products of stabilizers.

Table 1
Overview on the results of genotoxicity testing with "Arvin compounds" that migrate from thermally processed polyolefins as degradation products of stabilizers.

Substance	Mutagenicity *in vitro* (OECD TG 471)	Chromosome damage *in vitro* (OECD TG 487/473)	Chromosome damage *in vivo* (OECD TG 474)	Overall conclusion genotoxicity
Arvin 1 4-ethylphenol (CAS 123–07–9)	Negative	Positive	Negative (2 studies)	Negative
Arvin 2 *p-tert*-butylphenol (CAS 98–54–4)	Negative (2 studies)	Positive (1 study), Negative (1 study)[#]	Negative	Negative
Arvin 3 2,6-di-*tert*-butyl-p-benzoquinone (CAS 719–22–2)	Negative	Negative	No data available	Negative
Arvin 4 2,4-di-*tert*-butylphenol (CAS 96–76–4)	Negative (3 studies)	Positive (2 studies)	Negative[*,#]	Negative
Arvin 5 3,5-di-*tert*-butyl−4-hydroxystyrene (CAS 52858–87–4)	No data available			
Arvin 6 3,5-di-*tert*-butyl−4-hydroxybenzaldehyde (CAS 1620–98–0)	Negative	Negative	No data available	Negative
Arvin 7 3,5-di-*tert*-butyl−4-hydroxy acetophenone (CAS 14035–33–7)	Negative	Negative	No data available	Negative
Arvin 8 7,9-di-*tert*-butyl−1-oxaspiro(4,5)deca−6,9-diene−2,8-dione (CAS 82304–66–3)	Negative	Negative	No data available	Negative
Arvin 9 3-(3,5-di-*tert*-butyl−4-hydroxyphenyl)methyl propanoate (CAS 6386–38–5)	Negative	Negative	No data available	Negative
Arvin 10 3-(3,5-di-*tert*-butyl−4-hydroxyphenyl)propanoic acid (CAS 20170–32–5)	Negative	Negative	No data available	Negative

[*] Negative also in the Comet assay.
[#] Negative also in an additional Mouse lymphoma assay.

of S9. Increases in the number of revertant colonies above the threshold for considering a positive response (≥ 2-fold of the negative control value) were not observed in the test strains at any concentration of 4-ethylphenol applied. The positive controls gave the expected responses.

3.1.2. Genotoxicity assay in mammalian cells

4-Ethylphenol was assessed for the ability to induce chromosomal aberrations in Chinese hamster lung cells (CHL/IU) (FDSC 2000c). The experiments were performed according to OECD TG 473. 4-Ethylphenol (dissolved in DMSO) was tested at three concentrations, using short-term treatment in the absence and presence of metabolic activation (S9), for which incubations were conducted with six hour exposure followed by 18 hour culture prior to the analyses, or continuous treatment using a 24 hour exposure period in the absence of S9. Due to cytotoxicity in the cell cultures, the concentrations of 4-ethylphenol at which chromosome analysis was possible were limited to a maximum of 200 mg/L for the six hour exposures (without S9) and to 75 mg/L in the presence of S9 and the 24 hour exposures (without S9). In the study, 4-ethylphenol induced statistically significant increases (up to 3-fold) in the frequency of chromosome aberrations at 38 and 75 mg/L after the six hour exposure with S9 and the 24 hour exposure without S9. The results of this experiment indicate that 4-ethylphenol might induce structural chromosomal aberration in this cell type.

3.1.3. Assessment of genotoxicity in vivo

To clarify the relevance of the positive response of 4-ethylphenol in the *in vitro* chromosome aberration assay in mammalian cells, a mouse bone marrow micronucleus test (Verbaan, 2017) and a rat peripheral blood micronucleus test were conducted with 4-ethylphenol (ECHA, 2022). Both studies were performed following OECD TG 474 and included appropriate positive controls.

In the mouse bone marrow micronucleus test, mice (five/dose group) received two oral doses of 4-ethylphenol in corn oil (0, 125, 250 and 500 mg/kg bw/day in males and 0, 187, 375 and 750 mg/kg bw/day in females) by gavage within a 24 hour interval (Verbaan, 2017). The top doses that could be applied were limited due to toxicity as evident by mortality at higher doses in a preliminary dose range finding study. The positive control animals received a single dose of 40 mg/kg bw of cyclophosphamide, also by gavage. Bone marrow was sampled 48 hours after the first dosing. In the main test, males and females at the highest dose level showed clear signs of toxicity (such as lethargy, rough coat and hunched posture) and to some extent these clinical symptoms were also observed in females of the mid dose (375 mg/kg bw). Neither males nor females showed significant increases in the mean frequency of micronucleated polychromatic erythrocytes in the bone marrow or a decrease in the ratio of polychromatic to normochromatic erythrocytes. Administration of cyclophosphamide induced the expected statistically significant increase in the number of micronucleated polychromatic erythrocytes and a decrease in the ratio of polychromatic to normochromatic erythrocytes.

To demonstrate systemic exposure to the test item, plasma samples collected from an independent study conducted with the same strain of mice and using identical dosing conditions were analyzed for the presence of 4-ethylphenol. Blood samples were collected at 1, 2, 4 and 6 h after the second dosing and at 48 h following the first dosing just before bone marrow sampling. The analytical procedure applied was LC-MS/MS with limited sensitivity (limit of quantitation of 2 mg/L). In the samples from males dosed with 500 mg/kg bw, 4-ethylphenol could be detected at concentrations of 3.66 and 2.34 mg/L at 2 and 6 hours after dosing, respectively. In samples from females dosed with 750 mg/kg bw, the presence of 4-ethylphenol could be demonstrated at concentrations between 2.64 and 40.6 mg/L shortly (one and two hours) after the second dosing. In samples collected 24 h after the second dosing the concentration of 4-ethylphenol was below the lower limit of quantification (LLOQ) of 2 mg/L (Korsten, 2017; Verbaan, 2017). Despite the low sensitivity of the analytical assay, the presence of 4-ethylphenol in plasma indicates systemic exposure. The low concentrations found and the presence of 4-ethylphenol only in samples collected shortly after the last dosing indicate a rapid elimination of 4-ethylphenol from blood or

limited oral bioavailability due to first pass biotransformation. Phenols are subject to extensive first pass biotransformation after oral administration and formed glucuronides/sulfates are excreted rapidly (for overview, see (NTP, 2008)).

Based on the results of this study, it is concluded that 4-ethylphenol is not clastogenic or aneugenic in the mouse bone marrow micronucleus test.

The REACH registration dossier of 4-ethylphenol also includes results of a rat peripheral blood micronucleus assay. In this study, five male rats per dose group (or seven at the highest dose level) were administered 4-ethylphenol by gavage at single doses of 0, 125, 250, and 500 mg/kg bw in corn oil. The top dose of 500 mg/kg bw was considered the maximum tolerable dose based on adverse effects in dose range finding studies. Peripheral blood cells were harvested 48 hours after dosing (all groups) and 72 hours after dosing for controls and the 500 mg/kg bw group. Rats exposed to the two lower doses did not exhibit clinical signs, but clear indications of systemic toxicity were observed in the 500 mg/kg bw group including reduced activity, piloerection, ataxia and hunched posture up to four hours after administration. Increases in the frequency of micronucleated polychromatic erythrocytes in peripheral blood cells were not observed, supporting the conclusion that 4-ethylphenol does not induce structural and/or numerical chromosomal damage in the immature erythrocytes of the rat under the conditions tested.

Based on the negative results in bacterial mutagenicity testing and the two *in vivo* assays 4-ethylphenol is not considered genotoxic. Also, significant cytotoxicity occurred in the cell line used for the chromosome aberration assay (FDSC 2000c). Cytotoxicity is known to interfere with assessment of chromosome aberrations. Moreover, the Chinese hamster lung cells (CHL/IU) used are known to be p53-deficient. As p53-deficiency is discussed to be a key factor for false positive results in *in vitro* assays (Fowler et al. 2012), the biological relevance of the positive response of 4-ethylphenol in the *in vitro* clastogenicity assay remains questionable.

3.2. *p-tert-Butylphenol (CAS 98–54-4), „Arvin 2“*

For this compound, a number of *in vitro* and *in vivo* studies to assess potential genotoxicity is available. The available experiments include two independently performed bacterial reverse mutation assays, two assays for chromosome aberration and one for gene mutations in mammalian cells, and an *in vivo* mouse bone marrow micronucleus test.

3.2.1. *Bacterial mutagenicity assays*

Two studies assessing the mutagenicity of *p-tert*-butylphenol in bacteria are available conducted according to OECD TG 471 (FDSC 2000b; Jenkinson 1992c). Both studies used the plate incorporation method, tested with and without S9, and included the five test strains recommended by the OECD TG (*S. typhimurium* TA100, TA1535, TA98, and TA1537 and *E. coli* WP2 *uvrA*). The assays applied up to seven concentration levels of *p-tert*-butylphenol dissolved in DMSO and included the appropriate positive controls. Concentrations that could be used were limited by bacterial toxicity and the highest concentrations applicable without interfering bacterial toxicity were between 500 and 1000 µg/plate. In each study, the main test was repeated in a second experiment using triplicate cultures in either experiment. Relevant increases in the numbers of revertant colonies were not observed in both studies for the *p-tert*-butylphenol-treated cultures and the positive controls showed the expected increases in revertants.

3.2.2. *Genotoxicity assays in mammalian cells*

The available *in vitro* genotoxicity assays for *p-tert*-butylphenol include a clastogenicity assay with Chinese hamster lung cells (CHL/IU) (FDSC 2000d), a chromosome aberration assay in rat lymphocytes (Jenkinson 1992b), and a mouse lymphoma thymidine kinase mutation assay (Jenkinson 1992a).

In the clastogenicity assay in hamster lung cells, conducted according to OECD TG 473, *p-tert*-butylphenol was applied in DMSO at concentrations of up to 50 mg/L for 24 and 48 h exposures (without S9) and the six hour exposures with S9; six hour exposures without S9 were performed at concentrations up to 80 mg/L. Chromosome analysis was performed after 24 and 48 h (samples without S9), and 24 h after the start of the short-term exposure (6 h) to *p-tert*-butylphenol. In cultures exposed to *p-tert*-butylphenol in the presence of S9 for six hours, a concentration-dependent increase in the number of cells with structural aberrations and the number of polyploid cells was observed, while no significant increase in structural chromosomal aberrations occurred in the cells exposed continuously for 24 and 48 hours in the absence of S9. However, in these samples, a concentration-dependent increase in the frequency of polyploid cells was observed.

The clastogenic potential of *p-tert*-butylphenol was also evaluated in rat lymphocytes in two independent experiments performed following OECD TG 473. Lymphocyte cultures were exposed to three or four concentrations of *p-tert*-butylphenol dissolved in DMSO (up to a maximum of 62.5 mg/L) both in the presence and the absence of S9. Applicable concentrations were limited by cytotoxicity of *p-tert*-butylphenol. In the presence of S9, cultures were exposed for four hours; incubations in the absence of S9 lasted for 20 hours. Cells were harvested at 20 or 30 hours after the end of exposure with S9 and 20 hours without S9. Significant increases in the frequency of aberrations were not noted in the experiments with *p-tert*-butylphenol, but the positive controls (ethyl methane sulfonate and cyclophosphamide) showed the expected increases in aberration frequency.

Further, the ability of *p-tert*-butylphenol to induce mutations in mammalian cells was assessed in the mouse lymphoma/thymidine kinase assay following OECD TG 476 in two independent experiments (Jenkinson 1992a) applying five concentrations of *p-tert*-butylphenol in DMSO up to 80 mg/L (and up to 60 mg/L in the second experiment) both in the presence and the absence of S9. Applicable concentrations were again limited by cytotoxicity and ethyl methane sulfonate (- S9) and cyclophosphamide (+ S9) served as positive controls. In this experiment, *p-tert*-butylphenol did not increase the frequency of mutants at the TK +/- locus; but the positive controls induced the expected significant increases in mutant frequencies.

3.2.3. *Assessment of genotoxicity in vivo*

Due to the inconsistent results in the clastogenicity assays *in vitro*, possible cytogenetic effects of *p-tert*-butylphenol were evaluated in a mouse bone marrow micronucleus test according to OECD TG 474 using three dose levels of *p-tert*-butylphenol (12.5, 25, and 50 mg/kg bw) in 0.5 % Methyl cellulose by a single intraperitoneal administration to male mice (FDSC, 2002). Cyclophosphamide (50 mg/kg bw, by gavage) was used as positive control. The highest dose of *p-tert*-butylphenol was selected based on a range finding study showing mortality at doses of > 50 mg/kg bw. In all animals of the mid and high dose group (25 and 50 mg/kg bw), decreased locomotor activity was observed for one hour after dosing. This disappeared at six hours post-dose. Bone marrow smears were prepared 24 and 48 hours after administration of *p-tert*-butylphenol. Statistically significant increases in the frequency of micronuclei were not observed at both time points and significant differences in the proportion of polychromatic erythrocytes among total erythrocytes between the negative control group and the *p-tert*-butylphenol group did also not occur. Blood concentrations of *p-tert*-butylphenol were not assessed; however, the observed signs of toxicity can be considered as clear indication of systemic availability of *p-tert*-butylphenol.

In summary, the absence of a mutagenic response of *p-tert*-butylphenol in bacteria and mammalian cells and the negative bone marrow micronucleus test support the conclusion that *p-tert*-butylphenol is not mutagenic and not genotoxic.

3.3. 2,6-Di-tert-butyl-p-benzoquinone (CAS 719–22-2), "Arvin 3"

Genotoxicity of this compound is assessed based on an available bacterial reverse mutation assays and chromosome aberration assay.

3.3.1. Mutagenicity assay in bacteria

The mutagenicity of 2,6-di-*tert*-butyl-*p*-benzoquinone was assessed in the bacterial reverse mutation assay testing four strains of *S. typhimurium* (TA1535, TA1537, TA98, and TA100) and *E. coli* WP2 *uvrA* in the absence and presence of metabolic activation (S9). The study was conducted following OECD TG 471 and included the appropriate positive and negative controls (Verspeek-Rip 2016a). Five concentrations of 2,6-di-*tert*-butyl-*p*-benzoquinone dissolved in ethanol up to the maximum recommended concentration of 5 mg/plate were applied in triplicates in two independent experiments using modified parameters. At the top concentrations, 2,6-di-*tert*-butyl-*p*-benzoquinone precipitated on the plates, but only limited bacterial toxicity was observed. 2, 6-Di-*tert*-butyl-*p*-benzoquinone did not induce a significant or concentration-dependent increase in the number of revertants in the strains used, but the positive controls induced the expected increases in the revertant frequencies.

3.3.2. Genotoxicity assay in mammalian cells

The possible clastogenicity of 2,6-di-*tert*-butyl-*p*-benzoquinone was assessed in two independent experiments in cultured peripheral human lymphocytes both in the presence and absence of a metabolic activation system (S9) (Verbaan 2016a) in agreement with OECD TG 487. Lymphocytes were exposed to three concentrations of 2,6-di-*tert*-butyl-*p*-benzoquinone dissolved in ethanol (up to 80 mg/L) for three hours in the absence and up to 90 mg/L in the presence of S9 in an initial experiment. Cells were harvested after 27 hours. In the second experiment three concentrations of up to 30 mg 2,6-di-*tert*-butyl-*p*-benzoquinone/L were applied for 24 hours in the absence of S9, cells were harvested after another 24 hours. In control cultures, the number of mono- and binucleated cells with micronuclei were with the range of the historical controls and the positive controls mitomycin C and cyclophosphamide induced a statistically significant increase in the number of binucleated cells with micronuclei; colchicine produced a statistically significant increase in the number of mononucleated cells with micronuclei demonstrating that the test system provided valid results. In these experiments, 2,6-di-*tert*-butyl-*p*-benzoquinone was tested up to the recommended toxicity limit, producing cytotoxicity of 55 ± 5 % at the highest concentration while it did not induce increases in the frequency of micronuclei in the absence or presence of metabolic activation.

In summary, 2,6-di-*tert*-butyl-*p*-benzoquinone is neither mutagenic in bacteria nor clastogenic or aneugenic in mammalian cells.

3.4. 2,4-Di-tert-butylphenol (CAS 96–76-4), "Arvin 4"

For this compound, a number of experiments addressed endpoints relevant for assessing genotoxicity, including three bacterial reverse mutation assays, two chromosome aberration assays, as well as an *in vivo* micronucleus test and *in vivo* Comet assay.

3.4.1. Mutagenicity assays in bacteria

All three of the bacterial reverse mutation assays were performed in accordance with OECD TG 471 using the recommended combination of strains of Salmonella, *S. typhimurium* TA 1535, TA 98, TA 100, and TA1537 or TA97a (Andres, 2015; RIAS 2020a; Schöberl, 1991). One of the experiments also included *S. typhimurium* TA 102 and another included *E. coli* WP2 *uvrA*. Both, the plate incorporation and the preincubation method were applied in the absence or presence of metabolic activation (S9). Bacterial toxicity of 2,4-di-*tert*-butylphenol dissolved in DMSO was reported in all studies under conditions with and without S9 at concentrations ranging from > 375 μg/plate (Andres, 2015) to as low as ≥ 12.5 μg/plate in certain strains (RIAS 2020a). In one of the studies, the occurrence of precipitation was also reported at 500 μg/plate under preincubation conditions. However, relevant increases in the frequency of revertant colonies were not observed in any of the experiments, while the positive controls applied induced the expected increases in revertant frequencies.

3.4.2. Genotoxicity assays in mammalian cells

In a chromosomal aberration assay in Chinese hamster lung cells (V79) performed according to OECD TG 473, cells were exposed to three concentrations of 2,4-di-*tert*-butylphenol in acetone both in the presence and absence of S9. At 18 and 26 hours after the beginning of exposure, cells were arrested in metaphase, mitotic indices were determined, and metaphase cells were analyzed for chromosomal aberrations. Mitomycin C (without S9) and Cyclophosphamide (with S9) were used as positive controls and induced the expected increases in the frequencies of chromosomal aberrations. 2,4-di-*tert*-butylphenol was highly cytotoxic and applicable concentrations were limited to a maximum of 10 mg/L without S9 and 6 mg/L (5 mg/L in the second experiment) with S9. In two independent assays, increases in the frequencies of cells with chromosomal aberration were not observed without S9, but 2,4-di-*tert*-butylphenol induced significant increases in the frequency of cells with chromosomal aberrations in the highest concentrations in the presence of S9. The positive response however was only observed at concentrations inducing high cytotoxicity, i.e. relative mitotic index of 44.1–22.4 % of the control group. Hence, according to the study report, and as indicated in the respective OECD TG, the increased frequencies of cells with chromosomal aberration may be confounded by the high cytotoxicity of 2,4-di-*tert*-butylphenol (Krüger, 1998).

A second clastogenicity assay with 2,4-di-*tert*-butylphenol assessed the potential to induce chromosomal aberration in a Chinese hamster lung cell line (CHL/IU) (RIAS 2020b). Again, 2,4-di-*tert*-butylphenol dissolved in DMSO was highly cytotoxic with a reduction in cell growth of more than 50 % at 60 mg/L without S9 and at 10 mg/L with S9 in the dose range finder, limiting the highest concentration applied for the main study. Concentrations in the assay of chromosomal aberrations during short-term treatment (six hours followed by 18 hours culture) were therefore set to 10, 20, 30, 40, 50, and 60 mg 2,4-di-*tert*-butylphenol/L (without S9) and 1.25, 2.5, 5, 7.5, and 10 mg 2,4-di-*tert*-butylphenol/L (with S9). Increased frequencies of chromosomal aberrations were not observed in the absence of metabolic activation, but a concentration-dependent increase in the frequencies of cells with structural chromosomal aberrations was observed at 7.5 and 10 mg/L at a cell growth rate of 45 and 30 %, respectively. As the positive responses were only observed at the high end of, or even exceeding the recommended cytotoxic range of 55 ± 5 %, care should be taken interpreting these results with regard to biological relevance. The positive controls 1-methyl-3-nitro-1-nitrosoguanidine and 3,4-benzo[*a*]pyrene induced increased frequencies of cells with chromosomal aberrations.

3.4.3. Assessment of genotoxicity in vivo

To clarify the relevance of the positive trend observed in the *in vitro* chromosome aberration assays with 2,4-di-*tert*-butylphenol, a bone marrow micronucleus test in rats was conducted according to OECD TG 474 as well as an *in vivo* mammalian alkaline Comet assay with 2,4-di-*tert*-butylphenol in agreement with OECD TG 489.

In the *in vivo* micronucleus test, the potential of 2,4-di-*tert*-butylphenol to induce chromosomal damage was evaluated in male and female CD rats following two oral doses at an interval of 24 hours (Innes, 2008). Based on the results of a preliminary dose range finding study at which excessive toxicity was observed, three groups of female rats were administered 2,4-di-*tert*-butylphenol in corn oil at dose levels of 200, 400 and 800 mg/kg/day and an additional group of male rats received 1000 mg/kg/day. Two control groups of CD rats were dosed orally with either the vehicle, 20 mL corn oil/kg/day, or the positive control cyclophosphamide at 50 mg/kg/day. The experimental schedule for the control groups followed that of the test item treated rats. Bone marrow

samples were taken 48 h after the initial (0 h) dose.

No micronucleus induction was detected in bone marrow erythrocytes of rats when tested up to the maximum tolerated dose of 1000 mg/kg/day in male and 800 mg/kg/day in females CD rats. Administration of the control substances conformed to the expected control ranges.

Blood concentrations of 2,4-di-*tert*-butylphenol were not assessed; however, there were clear indications for systemic availability of the substance as male and female animals in the high dose groups showed clinical signs from the second day, such as hunched posture, subdued behavior, piloerection, wet feces, and red discharge from the nose. Moreover, there were indications of bone marrow toxicity in the high dose males, as the proportion of polychromatic to normochromatic erythrocytes was decreased (PCE/NCE ratio: 0.43 compared to 0.56 in the vehicle control group).

In the *in vivo* Comet assay, tissues assessed were liver, duodenum, and glandular stomach taken from groups of five male and female rats administered two oral dose levels of 0, 200, 400 or 800 mg/kg bw/day 2,4-di-*tert*-butylphenol in corn oil separated by a 20 hour time interval (Dewhurst, 2021). Comet and plasma samples were taken four hours after the last dosing. The highest dose applicable was assessed in dose range finder experiments in accordance with the TG with the aim to select the maximum tolerated dose (MTD), defined as the dose inducing slight toxic effects. In the main study the animals of the high dose group showed decreased body weight gains and clinical signs demonstrating that the MTD was reached. The plasma levels of 2,4-di-*tert*-butylphenol were determined by a sensitive LC/MS-MS method and administration of 2,4-di-*tert*-butylphenol resulted in a dose-dependent increase in plasma levels of 2,4-di-*tert*-butylphenol at the time of sampling of tissues for Comet analysis (mean plasma concentration of 2.73 mg/L in females and 1.08 mg/L in males receiving two doses of 800 mg/kg bw). Ethyl methane sulfonate (single dose of 300 mg/kg bw) served as positive control. According to the available data, 2,4-di-*tert*-butylphenol did not induce a genotoxic response in liver, duodenum, and glandular stomach. Due to the negative results in these tissues, Comet analyses were not done in the other tissues sampled (ovary, testes). According to the study report, the statistically significant and dose-related decrease in duodenum and glandular stomach DNA migration in female rats is indicative of cell loss due to cytotoxicity and not due to crosslink inductions reducing DNA migration. The dose-related increases in plasma concentrations of 2,4-di-*tert*-butylphenol in plasma samples indicate systemic bioavailability of 2,4-di-*tert*-butylphenol and suggest the exposure of target tissue.

In summary, equivocal results regarding clastogenicity *in vitro*, absence of mutagenicity in bacteria, and the negative *in vivo* micronucleus and Comet test with sufficient evidence for exposure of the target organs permit the conclusion that 2,4-di-*tert*-butylphenol does not induce genotoxic effects *in vivo*. Likely, the pronounced cytotoxicity of 2,4-di-*tert*-butylphenol contributed to the observed increases in the frequencies of cells with chromosomal aberrations in the *in vitro* assays.

3.5. 3,5-Di-tert-butyl-4-hydroxybenzaldehyde (CAS 1620–98-0), "Arvin 6"

For this compound, the genotoxic potential is assessed by a bacterial reverse mutation assay (Verspeek-Rip 2016a) and *in vitro* micronucleus assay (Buskens 2016b).

3.5.1. Mutagenicity assay in bacteria

The bacterial mutagenicity of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde was assessed in the *S. typhimurium* reverse mutation assay using strains TA1535, TA1537, TA98 and TA100 and in *E. coli* (WP2 *uvrA*). The testing procedure followed the respective OECD TG 471 and two independent experiments were conducted in the presence and absence of S9 (Verspeek-Rip 2016b).

Based on observations in a dose range finding test, the maximum applicable concentration was limited by precipitation of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde dissolved in DMSO at concentrations > 512 μg/plate, bacterial toxicity was not observed. The potential mutagenicity of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde was assessed in triplicates at five concentrations up to 2500 μg/plate in the first and up to 1568 μg/plate in the second experiment using modified conditions. The increases in revertant colonies were as expected with the positive controls included in the study design. 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde did not induce a significant or dose-related increase in the number of revertants in any of the strains used, neither in the absence nor presence of S9.

3.5.2. Genotoxicity assay in mammalian cells

Potential formation of micronuclei by 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde in cultured peripheral human lymphocytes was assessed in the presence and absence of metabolic activation (+/- S9) in two independent experiments (Verbaan 2016b). Study design and procedures applied followed OECD TG 487. In the first experiment, 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde dissolved in DMSO was added to the cultures at three concentrations up to 52 mg/L for three hours in the absence and presence of S9 and cells were harvested after 27 hours. Concentrations applied were limited by precipitation at the top concentration. In the second assay, 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde was tested at three concentrations for 24 hours in the absence of S9, with the highest concentration (52 mg/L) exceeding the limit of solubility. Cells were harvested after 24 hours. The positive controls, mitomycin C (- S9) and cyclophosphamide (+ S9), both produced statistically significant increases in the frequency of binucleated cells with micronuclei. Colchicine produced a statistically significant increase in the frequency of mononucleated cells with micronuclei. 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde did not induce a statistically significant increase in the frequency of mono- and binucleated cells with micronuclei neither in the absence or presence of S9. As the respective positive controls gave the expected increase in micronucleus frequencies, the study was considered valid, and it can be concluded that 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde is negative in the mammalian cell micronucleus assay under the conditions tested.

Taken together, the lack of a mutagenic response of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde in the bacterial reverse mutation assay and the negative *in vitro* micronucleus assay allow the conclusion that 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde is not mutagenic or genotoxic.

3.6. 3,5-Di-tert-butyl-4-hydroxy acetophenone (CAS 14035–33-7), "Arvin 7"

The available studies for the assessment of the genotoxic potential for this compound comprise a bacterial reverse mutation assay as well as an *in vitro* micronucleus assay.

3.6.1. Mutagenicity assay in bacteria

The bacterial mutagenicity of 3,5-di-*tert*-butyl-4-hydroxy acetophenone was assessed in the *S. typhimurium* and *E. coli* strains using design and procedures recommended in the OECD TG 471 in the presence and absence of S9 for metabolic activation (Verspeek-Rip 2016c). The substance was dissolved in DMSO and tested in triplicates up to and beyond precipitating concentrations; precipitation was observed at 500 and 1600 μg/plate in the absence and presence of metabolic activation, respectively. In two independent experiments, 3,5-di-*tert*-butyl-4-hydroxy acetophenone did not induce an increase in the number of revertant in the absence or presence of S9. Appropriate positive controls included in the study design produced the expected increases in revertants.

3.6.2. Genotoxicity assay in mammalian cells

Potential effects of 3,5-di-*tert*-butyl-4-hydroxy acetophenone on micronucleus frequencies in cultured peripheral human lymphocytes were assessed according to OECD TG 487 in the presence and absence of

S9 in two independent experiments (Buskens 2016a). The top concentrations chosen were based on precipitation of 3,5-di-*tert*-butyl-4-hydroxy acetophenone in the culture medium. In the first cytogenetic assay, 3,5-di-*tert*-butyl-4-hydroxy acetophenone was applied dissolved in DMSO in the absence and presence of S9 in concentrations of up to 164 mg/L in duplicate cultures for three hours and cells were harvested after 27 hours. The second experiment applied 3,5-di-*tert*-butyl-4-hydroxy acetophenone in the absence of S9 in concentrations up to 164 mg/L for 24 hours, followed by direct harvesting of the cells. In concurrent negative controls, the mean number of mono- and binucleated cells with micronuclei found remained within the limits of the negative control database of the laboratory, the positive control substance mitomycin C and cyclophosphamide both produced statistically significant increases in the number of binucleated cells with micronuclei. In addition, colchicine produced a statistically significant increase in the number of mononucleated cells with micronuclei. 3, 5-Di-*tert*-butyl-4-hydroxy acetophenone did not induce a statistically significant increase in the number of mono- and binucleated cells with micronuclei in the absence or presence of metabolic activation.

Based on the negative bacterial reverse mutation assay and the negative *in vitro* micronucleus test, it can be concluded that this compound is not genotoxic.

3.7. 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (CAS 82304–66-3), "Arvin 8"

For this compound, the genotoxic potential can be assessed based on an bacterial reverse mutation assay and an *in vitro* micronucleus assay (Buskens 2016b).

3.7.1. Mutagenicity assay in bacteria

The bacterial mutagenicity of 7,9-di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione was assessed in the *S. typhimurium* and in the *E. coli* strains as recommended by OECD TG 471 in the presence and absence of metabolic activation (S9) (Verspeek-Rip 2016d).

Due to precipitation, the top concentration of 7,9-di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione was limited to 1600 μg/plate. In two independent experiments, 7,9-di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione dissolved in DMSO and tested in triplicates did not induce a significant increase in the number of revertant colonies in the absence or presence of metabolic activation. Solvent controls and the recommended positive controls included in the design produced the expected increases in revertants consistent with historical controls of the performing laboratory.

3.7.2. Genotoxicity assay in mammalian cells

Potential effects of 7,9-di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione on micronucleus frequencies in cultured human peripheral lymphocytes were assessed in the presence and absence of metabolic activation (S9) in two independent experiments (Buskens 2016b). The experiments were performed as recommended in OECD TG 487 and top concentrations were limited by precipitation and/or cytotoxicity of 7, 9-di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione. In the first cytogenetic assay, 7,9-di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2, 8-dione was tested in duplicates at concentrations up to 110 mg/L in the absence and of up to 164 mg/L in the presence of S9 for three hours and cells were harvested after 27 hours. Precipitation was observed at the top concentrations. The second experiment applied 7,9-di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione in duplicate cultures in the absence of S9 at concentrations up to 110 mg/L for 24 hours; cells were fixed directly after the end of exposure. In controls, the mean number of mono- and binucleated cells with micronuclei remained within the limits of the negative control database, the positive controls mitomycin C and cyclophosphamide both produced a statistically significant increase in the number of binucleated cells with micronuclei, and colchicine produced a statistically significant increase in the number of mononucleated cells with micronuclei. 7,9-Di-*tert*-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione did not induce a statistically significant increase in the number of mono- and binucleated cells with micronuclei in the absence or presence of metabolic activation.

In summary, based on the negative results in the bacterial reverse mutation assay and mammalian cell micronucleus assay, 7,9-di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione can be considered non-genotoxic.

3.8. 3-(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl propanoate (CAS 6386–38-5), "Arvin 9"

Genotoxicity of this compound is assessed by a bacterial reverse mutation assay (Deparde 1983b) and a chromosome aberration assay (Ogorek, 1994).

3.8.1. Mutagenicity assay in bacteria

The bacterial mutagenicity of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) methyl propanoate in acetone was assessed only in *S. typhimurium* (TA 98, TA 100, TA 1535, TA 1537, and TA 1538) in the presence and absence of metabolic activation (S9) at five concentrations up to 5120 μg/plate. Precipitation was observed at the highest concentration tested (Deparde 1983a). Appropriate positive and solvent controls were included in the study. The experiment was repeated for confirmation and all conditions were tested in triplicates in either experiment. 3-(3, 5-Di-*tert*-butyl-4-hydroxyphenyl)methyl propanoate did not induce a significant and concentration-related increase in the number of revertants in the absence or presence of S9. Positive controls included in the study design produced the expected increases in revertants.

3.8.2. Genotoxicity assay in mammalian cells

The endpoint clastogenicity of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) methyl propanoate was investigated using a Chinese hamster ovary cell line assessing chromosome aberrations both in the presence and absence of S9 in two independent experiments following OECD TG 473 (Ogorek, 1994). Due to cytotoxicity, concentrations of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl propanoate (in DMSO) suitable for evaluation were limited to a maximum of 19.5 mg/L in the absence and 156.3 mg/L in the presence of S9. Experiments with S9 were conducted with three hours exposure followed by 15 or 39 hours exposure-free incubation period prior to harvest of the cells for chromosome analyses. Incubations in the absence of S9 were performed with exposures for 18 and 42 hours, followed by direct harvest and analysis of cells. Mitomycin C and cyclophosphamide served as positive controls and resulted in an increased frequency of chromosomal aberrations. 3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)methyl propanoate did not lead to a biologically relevant increase of the frequency of chromosomal aberrations either in the presence or absence of metabolic activation.

Taken together, the results of the *in vitro* studies described above support the conclusion that 3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl) methyl propanoate is non-genotoxic.

3.9. 3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propanoic acid (CAS 20170–32-5), "Arvin 10"

The available studies for the assessment of the genotoxic potential for this compound comprise a bacterial reverse mutation assay (Deparde 1983a) and a chromosome aberration assay (Ogorek, 1992).

3.9.1. Mutagenicity assay in bacteria

The bacterial mutagenicity of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propanoic acid in acetone was assessed only in *S. typhimurium* (TA 98, TA 100, TA 1535, TA 1537, and TA 1538) in the presence and absence of metabolic activation at five concentrations up to 5120 μg/plate. Precipitation was observed at the two highest concentrations tested (Deparde 1983a). Appropriate positive and solvent controls were

included in the study design. The experiment was repeated for confirmation and all conditions were tested in triplicates in either experiment. 3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)propanoic acid did not induce a significant and concentration-related increase in the number of revertants in the absence or presence of metabolic activation. The positive controls included produced the expected increases in revertants.

3.9.2. Genotoxicity assay in mammalian cells

The potential clastogenicity of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propanoic acid was investigated in a Chinese hamster ovary cell line both in the presence and absence of S9 in two independent experiments (Ogorek, 1992). The study design followed OECD TG 473. Due to cytotoxicity, concentrations of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propanoic acid suitable for evaluation were limited to a maximum of 415 mg/L with S9 and 207.5 mg/L (18 h) or 103.8 mg/L (42 h) without S9; solvent was DMSO. Incubations with metabolic activation were conducted with three hours exposure followed by either 15 or 39 hours exposure-free time prior to harvest for chromosome analyses. Incubations in the absence of S9 were performed with exposure time of 18 or 42 hours, followed by direct harvest and analysis. Mitomycin C and cyclophosphamide served as positive controls and induced the expected increased frequency of chromosomal aberrations. 3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)propanoic acid did not cause a biologically relevant increase in the frequency of chromosomal aberrations either in the presence or absence of metabolic activation.

Based on the negative results in the bacterial reverse mutation assay and *in vitro* chromosome aberration assay, 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanoic acid can be considered non-mutagenic and non-genotoxic.

4. Genotoxicity of other alkylphenols

Alkylphenols such as chemicals represented by some Arvin compounds are widely used for different technical purposes and many representatives of this chemical class are also natural constituents of human diet (Api et al. 2021; EFSA, 2017). Therefore, results from the database on the genotoxicity of these chemicals can be integrated into the assessment of the potential genotoxicity of Arvin compounds.

A dedicated assessment of genotoxicity of a group of several branched chain alkylphenols including some Arvin substances has been performed (Matsumoto et al. 2011). Identical to the results of the Arvin substances summarized above, the alkylphenols assessed (*o-sec*-butylphenol, *p-sec*-butylphenol, 6-*tert*-butyl-m-cresol, and 2-isopropyl-5-methylphenol) showed consistently negative results in bacterial mutagenicity, inconsistent responses in *in vitro* chromosome aberration assays, and negative responses in *in vivo* micronucleus assays. Identical conclusions were made by EFSA in the evaluation of alkylphenols that are present in a variety of flavorings. In the large series of alkylphenols considered, negative results were quite consistently obtained in the more reliable studies with the endpoint bacterial mutagenicity; however, results in *in vitro* chromosome aberrations assays were inconsistent between studies and alkylphenol compounds, with positive, equivocal, and negative results. The results from the more reliable assays were considered negative (EFSA 2011b). Based on these observations, EFSA concluded on no safety concern regarding human health at the estimated levels of intake in the low micrograms/kg bw/day range. Similarly, the widely used cresol isomers, *o*-cresol, *m*-cresol and *p*-cresol (as well as a mixture "*m*-/*p*-cresol") are negative in bacterial mutagenicity assays and *o*-cresol and *m*-/*p*-cresol do not induce micronuclei in erythrocytes of mice after dietary exposures for 13 weeks (NTP, 2008).

5. Summary conclusions

Results from experimental genotoxicity testing addressing mutagenicity and clastogenicity are available for nine out of the ten Arvin substances. All Arvin substances tested were consistently negative in bacterial mutagenicity studies, but inconsistent or positive results were obtained in clastogenicity assays in cultured cells for three of the Arvin compounds. Regarding the endpoint clastogenicity, the alkylphenols Arvin 1, 2, and 4 induced positive or equivocal responses in the presence of metabolic activation at concentrations that induced significant cytotoxicity. Neither of these compounds induced a positive response in *in vivo* micronucleus tests and Arvin 4 was also negative when tested in a Comet assay. This response pattern of Arvin 1, 2, and 4 in clastogenicity assays *in vitro* is similar to that observed with a number of other alkylphenols which also gave both positive and negative responses in cultured cells. With Arvin 1, 2, and 4, pronounced cytotoxicity in cultures of mammalian cells may be a contributing factor as the high rates of false positive results in clastogenicity assays may be due to cytotoxicity/apoptosis (Kirkland et al. 2007; Meintières and Marzin, 2004; Nesslany, 2017), but other direct and indirect effects may also be relevant under the conditions of the *in vitro* assays. For example, alkylphenols may interfere with microtubule assembly (Pfeiffer et al. 1997), oxidative damage may be induced by alkoxy radicals (Bolton, 2014), and quinone methides may be formed by enzymatic oxidation (Thompson et al. 1995). However, metabolic processes may widely differ between *in vitro* assays and the *in vivo* situation. For example, S9 used in cell cultures for simulation of biotransformation well simulates oxidative bioactivation by cytochromes P450 (Kirkland et al. 2007; Nesslany, 2017), but is deficient in the capacity for conjugation of xenobiotics with glucuronic acid and sulfate. Conjugation with glucuronic acid and/or sulfate are major processes that efficiently convert phenols and alkylphenols to excretable metabolites (Cheetham and Zwarenstein, 1938).

In case of inconsistent *in vitro* genotoxicity, appropriate *in vivo* short-term test methods need to be applied to support a conclusion on genotoxicity. For Arvin 1, 2, and 4, such studies are available and resulted in negative results supporting the conclusion that these compounds are not genotoxic. This assessment is in line with EFSAs conclusion on the absence of a genotoxic risk due to the presence of alkylphenols in flavorings. The other Arvin compounds consistently did not show responses in bacterial mutagenicity and assays for clastogenicity. In conclusion, regarding human health risk characterization, Arvin compounds (except Arvin 5 for which genotoxicity studies were not considered necessary) are not considered genotoxic agents based on the available data.

Declarations

The author certifies that his freedom to design, conduct, interpret, and publish research was not compromised by the sponsor. No competing interests. The author was compensated for the time spent to produce this review at his usual hourly rate.

CRediT authorship contribution statement

Wolfgang Dekant: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Wolfgang Dekant reports financial support was provided by Elisana. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

No data was used for the research described in the article.

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