

Annex L. Weight of evidence on Genotoxicity

Annex to: Scientific Opinion on the Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal <https://doi:10.2903/j.efsa.2023.6857>

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Tables summarising new *in vitro* and *in vivo* genotoxicity studies on BPA identified in the literature (2013 – 2021) and studies considered in the 'Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs' (EFSA CEF Panel, 2015).

The studies have been evaluated based on the criteria described in Section 2.3.5 of the Scientific opinion.

*Indicates that more than one assay is reported/indicates when papers belong to more than one table.

**Indicates that both *in vitro* and *in vivo* assays are reported in the same paper.

Table 1: Bacterial reverse mutation assay
(OECD TG 471 was considered for the evaluation of reliability)

Test system/Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance	Results	Reliability/Comments	Relevance of the result	Reference (authors and RefID)
Bacterial reverse mutation assay <i>Salmonella</i> Typhimurium strains TA 98 and TA 100 <i>In vivo</i> micronucleus assay (Table 7)**	BPA 1–10 µmoles/plate with or without S9; 3 replicates	BPA (Tokyo Kasei Kogyo Co., Ltd) Purity 99% not reported in the study but available in the website of the company	Negative	Reliability: 2 Only 2 strains Data on negative controls subtracted (but not shown) No positive control	Limited	Masuda et al., 2005 ^{1**}
Bacterial reverse mutation assay <i>Salmonella</i> Typhimurium strains TA98, TA100, TA102 <i>In vivo</i> chromosomal aberration (Table 6) micronucleus assay (Table 7) comet assay (Table 8)**	BPA 0, 6.25, 12.5, 25, 50, 100, 150 and 200 µg/plate for 48 h; with or without S9; pre-incubation method	BPA, purity 99% (Sigma Chemical Company)	Negative	Reliability: 2 Only 3 strains used	Limited	Tiwari et al., 2012 ^{1**}
Bacterial reverse mutation assay <i>Salmonella</i> Typhimurium strains TA98 and TA 100 <i>In vitro</i> comet assay (Table 5)*	BPA 0, 4, 20, 100, 500 µg/plate for 48 h (TA100) and 72 h (TA98); 3 replicates; with or without S9	BPA, purity >99% (Sigma-Aldrich)	Negative	Reliability: 2 Only 2 strains	Limited	Fic et al., 2013 ^{1*}

¹ Studies considered in the *Scientific Opinion on the Risks to Public Health Related to the Presence of Bisphenol A (BPA) in Foodstuffs* (EFSA CEF Panel, 2015).

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Bacterial reverse mutation assay <i>Salmonella</i> Typhimurium strains TA1535, TA97, TA98, TA100 and TA102 <i>In vitro</i> chromosomal aberration (Table 3) micronucleus assay (Table 4) comet assay (Table 5) in CHO cells*	BPA 10–5000 µg/plate; 48 h incubation; with or without S9; pre-incubation method in triplicates; 3 independent experiments	BPA (purity 99%) ² , was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China)	Negative	Reliability: 1	High	Xin et al., 2015* [RefID 8150]
Bacterial reverse mutation assay <i>Salmonella</i> Typhimurium strains TA98 and TA100	BPA 0.1, 1, 10 and 100 µg/plate with or without S9; plate incorporation assay in triplicates; 2 independent experiments	BPA (Merck) Purity >97% not reported in the study but available on the website of the company	Negative	Reliability: 2 Only 2 bacterial strains used	Limited	Zemheri and Uguz, 2016 [RefID 9535]
SOS/umuC assay in <i>Salmonella</i> Typhimurium TA1535 pSK1002 <i>In vitro</i> comet assay (Table 5)*	BPA 0, 1, 10, 100, 1000 µg/L, without or with metabolic activation (S9)	BPA (Sigma-Aldrich) Purity >97% not reported in the study but available on the website of the company	Negative	Reliability: 2 Non-standard test applied as a preliminary analysis of toxicity and mutagenicity	Limited	Balabanič et al., 2021* [RefID 224-G]

² Information on BPA purity provided by the study authors on 11 October 2021, upon EFSA request

Table 2: *In vitro* gene mutation in mammalian cells

Test system/Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance	Results	Reliability/Comments	Relevance of the result	Reference (authors and RefID)
Analysis of mutational spectra in immortalised human embryonic kidney cells HEK 293T using whole genome sequencing (WGS) DNA double strand breaks as measured using γH2AX immunofluorescence staining	100 µM for 24 h exposure and WGS of clonally expanded cells populations No metabolic activation Cell viability analysed in HEK 293T cells, treated for 24 h with 0.1, 1 and 100 µM BPA; cells were stained with crystal violet and results reported as colony area percentage	BPA from TCI (B04 94) purity ≥ 99% not reported in the study but available on the website of the company	Positive Increased levels of single base substitutions, double-strand breaks and small insertions/deletions in BPA-treated HEK 293T cells in comparison with DMSO-treated controls Single base substitutions (C>A transversions) in BPA-treated cells preferentially occur at guanines Mutations at A:T bp were also reported Colony formation assay: concentration-dependent decrease in % colony area Concentration-dependent increase in DNA double strand breaks as increased number of nuclei with > 5 γH2AX foci	Reliability: 2 Although there is no TG for this type of study, the research was adequately conducted and reported However, there is uncertainty in the level of toxicity of the BPA treatment	Limited	Hu et al., 2021 [RefID 295-G]

Table 3: *In vitro* chromosomal aberrations test
(OECD TG 473 was considered for the evaluation of reliability)

Test system/Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance	Results	Reliability/Comments	Relevance of the result	Reference (authors and RefID)
Chromosomal aberrations and SCE assays CHO-K1 cell line Cytotoxicity: cell-cycle delay 'recognised by the metaphases without differently staining sister chromatids' <i>In vitro</i> comet assay (Table 5)*	BPA 0, 0.1 to 0.6 mM for 3 h followed by 27 h recovery 100 metaphases SCE: 50 metaphases Without metabolic activation	BPA, purity > 99% (Tokyo Kasei Kogyo Co., Ltd)	Positive Only in presence of severe cytotoxicity Increased CA (0.5, 0.55, 0.6 mM, % of differently staining sister chromatids 29%, 11%, and 0%, respectively) Increased endo-reduplications (0.45 and 0.55 mM) Increased frequency of c-mitosis-like figures (above 0.3 mM) Increased SCE (0.4 and 0.5 mM)	Reliability: 3 Only short-term treatment; high level of cytotoxicity The recovery time exceeded the recommended (18–21 h) Cells recovered in the presence of BrdU	Low	Tayama et al., 2008^{1*}
Chromosomal aberration assay CHO cells Cytotoxicity: MTT assay Bacterial reverse mutation assay (Table 1)	BPA 0, 80, 100 and 120 µM for 24 h 500 metaphases/group; without metabolic activation MTT assay: BPA 0, 40, 80, 100 and 120 µM for 12 and 24 h	BPA (purity 99%)², was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China)	Positive Increase of structural chromosomal aberrations from 80 µM, with significant decrease in cell viability (but not lower than 50%) MTT assay: increase of cell proliferation at 40 µM; cytotoxicity from 80 µM	Reliability: 2 No short-term treatment No positive control	Limited	Xin et al., 2015* [RefID 8150]

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<i>In vitro</i> micronucleus assay (Table 4), comet assay (Table 5)*						
Chromosomal aberration assay in: - MCF-7 human breast cancer line; - human amniocytes from male [oestrogen receptors (ER) negative] and from female (ER positive) Cytotoxicity: MTT test	BPA 0, 0.4, 1, 4, 40 and 100 µg/mL for 48 h; 200 cells analysed for each treatment (less at highest concentrations in amniocytes for high toxicity) Without metabolic activation MTT test: BPA 0, 0.4, 1, 4, 40, 100 and 400 µg/mL for 48 h.	BPA, no information on purity or the supplier company	Positive Increase of cells with chromosome aberrations (from 1 µg/mL) in all cell types; the increase in cells with aberrations was not clearly concentration related and decreased at the highest concentrations, possible due to cytotoxicity that was not concurrently evaluated; no clear association with ER expression In a preliminary evaluation of cytotoxicity by the MTT test, the IC ₅₀ of BPA was 100, 40 and 4 µg/mL in MCF-7 and ER-negative (male) and ER-positive (female) amniocytes, respectively	Reliability: 2 Cells scored less than recommended in OECD TG 473 No short-term treatment No positive control No concurrent control of toxicity	Low No information on source and purity of BPA	Aghajanpour-Mir et al., 2016 [RefID 57]
Chromosomal aberration assay Human peripheral blood lymphocytes from 5 female subjects <i>In vitro</i> micronucleus assay (Table 4)*	BPA 0, 0.20, 0.10, 0.05, 0.02 and 0.01 µg/mL for 24 h 1000 metaphases (200/subject)/concentration Without metabolic activation	BPA (Sigma-Aldrich) purity ≥97% not reported in the study but available on the website of the company	Positive Increase from 0.05 µg/mL (prevalence of chromatid breaks) No numerical aberrations	Reliability: 2 No short-term treatment	Limited	Santovito et al., 2018* [RefID 11220]
Chromosomal aberrations	BPA 150 µM for 24 h	BPA (Sigma-Aldrich) purity	Negative	Reliability: 3	Low	Sonavane et al., 2018* [RefID 419-G]

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Mouse embryonic fibroblasts (MEF) <i>In vitro</i> comet assay (Table 5)*	or co-exposure with camptothecin (CPT) 25 metaphases/treatment were analysed Without metabolic activation	≥97% not reported in the study but available on the website of the company	No significant increase in CA frequency Cytotoxicity of BPA alone was not measured but the authors refer to 150 µM as concentration with minimal toxic effect from a previous publication	Single concentration tested; low number of metaphases analysed No short-term treatment		
Chromosomal aberrations Human peripheral blood mononuclear cells (PBMC) Cell proliferation: MTT test Cell-cycle analysis: FACS γH2AX: western blot and FACS analysis	BPA 0, 25, 50, 100 nM, cells stimulated with PHA for 16h and then treated with BPA for 48 h 30 metaphases/treatment/subject (5 donors) MTT test: BPA 0, 5, 10, 25, 50, 100, 200 nM and BPA 25, 50, 100, 200 µM, cells were treated with or without PHA for 16 h and then treated with BPA for 24 and 48 h γH2AX: cells treated with PHA and then with BPA 50 nM for 24 h or 48 h (western blot) or only for 24 h (FACS analysis analysing T and B lymphocytes) Without metabolic activation	BPA (Merck) Purity ≥97% not reported in the study but available on the website of the company	Positive Increased number of aberrant cells, structural chromosomal aberrations and highly fragmented metaphases <u>MTT test:</u> - unstimulated PBMCs: decreased cell proliferation only at 200 µM at both 24 and 48 h PHA stimulated PBMCs: - increased cell proliferation from 10 nM to 100 nM; - concentration-dependent decreased cell proliferation from 25 to 200 µM Effect on cell proliferation confirmed using cell-cycle analysis γH2AX (western blot): - increase of protein phosphorylation only at 24 h (BPA 50 nM) γH2AX (FACS):	Reliability: 2 No positive control No short-term treatment	Limited	Di Pietro et al., 2020 [RefID 258-G]

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			increase in CD3 ⁺ and in CD4 ⁺ T cells			
Chromosomal aberrations assay in human peripheral blood lymphocytes	BPA 0, 5, 10, 20 and 50 µg/mL for 24 and 48 h Mitomycin C (MMC) at 0.10 µg/mL 'was added to the negative and a positive controls and to each concentration and chemical groups as well' Without metabolic activation	BPA, no information on purity or the supplier company	No data on chromosome aberrations were reported	Reliability: 3 MMC added to all treatments No mitogenic stimulation No short-term treatment	Low No information on BPA purity	Özgür et al., 2021 [RefID 631-G]

Table 4: *In vitro* mammalian cell micronucleus test
(OECD TG 487 was considered for the evaluation of reliability)

Test system/Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance	Results	Reliability/Comments	Relevance of the result	Reference (authors and RefID)
Cytokinesis block micronucleus assay (CBMN) AHH-1 cell line (human lymphoblastoid cells) Effects on mitotic spindle using staining: brilliant blue and safranin O; α- and γ-tubulin immunofluorescence staining	BPA 0, 1.5, 3.1, 6.2, 7.7, 9.2, 10.8, 12.3, 18.5, 24.6, 37 $\mu\text{g/mL}$ for a complete cell cycle (22–26 h), Five experiments: average of 8082 cells scored for each treatment Effects on mitotic spindle: BPA 0, 4.2–14 $\mu\text{g/mL}$ for 20 h (one cell cycle); 100 cells undergoing mitosis scored in each experiment, 3 experiments Without metabolic activation	BPA (Sigma-Aldrich) purity $\geq 97\%$ not reported in the study but available on the website of the company	Positive increased BNMN cells from 12.3 $\mu\text{g/mL}$ Aberrant mitotic divisions (multiple spindle poles)	Reliability: 1 BN cells % as parameter of cytotoxicity High number of analysed binucleated cells	High	Johnson and Parry, 2008¹
Micronucleus test in: - human umbilical vascular endothelial cells (HUVEC); - human colon adenocarcinoma (HT29) cell line Immunofluorescence analysis of cytoskeleton organisation of HUVEC	BPA 0, 44 nM and 4.4 μM, (i.e. 10 ng/mL and 1 $\mu\text{g/mL}$) for 72 h BPA 10 ng/mL and 1 $\mu\text{g/mL}$ for 24 or 72 h CellTiter-Blue assay: BPA 10 ng/mL and 1 $\mu\text{g/mL}$ for 24, 48 or 72 h	BPA, no information on purity or the supplier company	Positive in HUVEC cells: slight increase of MN frequency Negative in HT29 cells Multipolar spindles and microtubule misalignment	Reliability: 2 No analysis of cell proliferation; no positive control; no short-term treatment	Low No information on source and purity of BPA	Ribeiro-Varandas et al., 2013 [RefID 6189]

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cells with anti- α -tubulin and anti- γ -tubulin Apoptosis using TUNEL assay and cell viability using CellTiter-Blue assay	Without metabolic activation		associated with BPA exposure No effects on cell viability, proliferation and apoptosis in both cell lines			
Cytokinesis block micronucleus assay; bovine peripheral blood lymphocytes; cell proliferation: nuclear division index (NDI)	BPA 1×10^{-4} , 1×10^{-5} , 1×10^{-6} and 1×10^{-7} mol/L for 48 h Without metabolic activation	BPA (Sigma-Aldrich) Purity $\geq 97\%$ not reported in the study but available on the website of the company	Positive concentration-related increase in MN frequency, statistically significant at the highest concentration; no effect on NDI at any concentration	Reliability: 2 No short-term treatment; bovine lymphocytes are not commonly used in the micronucleus test, and their use has not been validated. However the study appears to be adequately performed and reported	Limited	Šutiaková et al., 2014 [RefID 7026]
Micronucleus assay CHO cells Cytotoxicity: MTT test Bacterial reverse mutation assay (Table 1) <i>In vitro</i> chromosomal aberration (Table 3) comet assay (Table 5)*	BPA 0, 80, 100 and 120 μ M for 24 h, without cytochalasin B; 1000 cells were scored for each sample; 3 independent experiments Without metabolic activation MTT test: - BPA 0, 40, 80, 100 and 120 μ M for 12 and 24 h	BPA (purity 99%) ² , was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China)	Positive increase in MN frequency at 100 and 120 μ M MTT assay: concentration-related decrease in cell viability from 100 μ M	Reliability: 2 No short-term treatment No positive control	Limited	Xin et al., 2015* [RefID 8150]
Cytokinesis-blocked micronucleus assay in murine macrophage RAW264.7 cells 1000 binucleated cells/concentration	BPA 0, 3, 10, 30, or 50 μ M for 24 h BPA 10 μ M tested for MN assay and cell viability, in the presence or absence of pre-	BPA (Sigma-Aldrich) Purity $\geq 97\%$ not reported in the study but available on the website of the company	Positive Concentration-dependent increase in MN frequency from 10 μ M	Reliability: 2 No short-term treatments; no positive controls; no data on cell proliferation	Limited	Huang et al., 2018* [RefID 296-G]

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Cell viability: MTT test <i>In vitro</i> comet assay (Table 5)*	treatment with <i>N</i> -acetyl-L-cysteine (NAC) at the concentration of 10 µM for 30 min Without metabolic activation MTT test: BPA 0, 3, 10, 30, or 50 µM for 12 or 24 h		In the presence of NAC, MN frequency and cytotoxicity were statistically significantly reduced (see also data on ROS in Table 5) MTT test: concentration- and time-dependent decrease of cell viability			
Cytokinesis block micronucleus assay Human peripheral blood lymphocytes from 5 female subjects 1000 binucleated lymphocytes/subject (5000 binucleated cells per concentration) <i>In vitro</i> chromosomal aberrations assay (Table 3)*	BPA 0, 0.20, 0.10, 0.05, 0.02 and 0.01 µg/mL for 48 h Without metabolic activation	BPA (Sigma-Aldrich) Purity ≥97% not reported in the study but available on the website of the company	Positive Increase in MN frequency from 0.02 µg/mL. At 0.2 µg/mL 4-fold increase with respect to the vehicle control (DMSO) level No significant reduction of the CBPI value	Reliability: 2 No short-term treatment	Limited	Santovito et al., 2018* [RefID 11220]
Mitotic abnormalities and micronuclei evaluated in DAPI stained cells: - Hep-2 cells (human epithelial cells from laryngeal carcinoma); - MRC-5 cells (human lung fibroblasts) Cell viability using CellTiter-Blue assay, after 48 h exposure	BPA 0.44 nM, 4.4 nM, 4.4 µM (0.1 ng/mL, 1 ng/mL, 1 µg/mL) for 48 h; 1000 cells scored for each treatment	BPA (Sigma) purity ≥97% not reported in the study but available on the website of the company	Positive Slight (two-fold) increase in MN frequency from BPA 4.4 nM in both cell lines Mitotic index: - in Hep-2 cells, no effects;	Reliability: 3 No short-term treatment Proliferation of the cell population not determined; extremely low % of mitosis is indicative of a very low rate of cell division, which is not	Low	Ramos et al., 2019* [RefID 396-G]

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<i>In vitro</i> comet assay (Table 5)*			- in MRC-5 cells, statistically significant increase Cytotoxicity: no effects on cell viability	appropriate to measure MN formation Protocol of MN assay not reported; no positive control		
Micronucleus assay in Chinese hamster V79-derived cell lines expressing various human CYP enzymes Micronucleus assay in C3A cells (human hepatoma cell line, endogenously express various CYP enzymes, including CYP1A1, 1A2, 1B1, 2E1, 3A4, and phase II metabolic enzymes, such as UGTs and SULTs) 2000 cells analysed for each treatment Cytotoxicity: CCK-8 Assay γ-H2AX in V79-Mz, V79-hCYP1A1 cells and in C3A cells; analysis using In-Cell Western Blot Immunofluorescence staining of CENP-B of MN induced in C3A cells	1) Micronucleus assay in V79-derived cell lines: - BPA 0, 40, 80, 160 µM for 9 h + 15 h; (recovery period); - 2000 cells analysed for each treatment 2) Micronucleus assay in: - V79-Mz, V79-hCYP1A1 cells: BPA 0 to 80 µM for 24 h + 0 h ; with or without ABT; - C3A cells: BPA 0 to 80 µM for 72 h + 0 h; with or without ABT or 7-HF 3) Micronucleus assay in C3A cells: BPA 0 to 5 µM for 72 h + 0 h, with or without KET or PCP (phase II enzyme inhibitors), an inhibitor of UGT1 and SULT1, respectively Immunofluorescence staining of CENP-B was applied Cytotoxicity performed for each test using the	BPA (99.6%), AccuStandard Inc.	1) Micronucleus assay (9 h + 15 h): - Negative in V79-Mz; - Positive in V79-hCYP1A1 cells and in V79-hCYP1B1 cells; - Cytotoxicity: statistically significant decrease at the highest concentrations 2) Micronucleus assay (24 h + 0 h): - Negative in V79-Mz; - Positive in V79-hCYP1A1 cells, effect abrogated by ABT 2) Micronucleus assay (72 h + 0 h): - Positive in C3A cells, effect abrogated by ABT or 7-HF; - Cytotoxicity: statistically significant decrease at the highest concentrations	Reliability: 2 Micronucleus method poorly described No short-term treatment	Limited	Yu et al., 2020 [RefID 475-G]

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	same testing conditions of the MN assay or of γH2AX analysis γH2AX: BPA 0, 10, 20, 40, 80, 160 μM for 9 h; ABT (1-aminobenzotriazole a CYP inhibitor) or 7-HF (a selective CYP1A1 inhibitor) were added from 2 h ahead of test compound exposure to the end of cell culture		3) Micronucleus assay in C3A cells (72 h + 0 h): - Positive - Effects enhanced by KET or PCP; statistically significant increase of MN negative for CENP-B staining, (clastogenic mechanism) Cytotoxicity: statistically significant increase in cell viability from 2.5 μM γH2AX: - increase in V79-Mz, in V79-hCYP1A1 cells and in C3A cells (concentration dependent); effect reduced by ABT or 7-HF			
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Table 5: *In vitro* DNA damage (comet assay)

Test system/Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance	Results	Reliability/Comments	Relevance of the result	Reference (authors and RefID)
Alkaline comet assay MCF-7 (oestrogen receptor (ER) positive) and MDA-MB-231 (ER negative) γH2AX foci using immunofluorescence in MCF-7 cells	MCF-7 cells exposure: - BPA 0, 0.1 10, 100 μM for 3 h; - BPA 100 μM for 1, 3, 24 h MDA-MB-231 cells exposure: - BPA 100 μM for 3, 24 h; 30 cells analysed (10 cells/slide) Immunofluorescence in MCF-7 cells: BPA 10 μM for 3 h Without metabolic activation	BPA (Wako Pure Chemicals Industries, Ltd) purity ≥99% not reported in the study but available on the website of the company	Positive MCF-7: increased comet tail length after 3 h at 10, 100 μM and after all exposure times at 100 μM MDA-MB-231: increased comet tail length after 3 and 24 h exposure times at 100 μM No toxicity in comet assays Induction of γH2AX foci in MCF-7 cells (10 μM) ER-positive MCF-7 cells are more sensitive than ER-negative MDA-MB-231 cells to BPA-induced DNA damage	Reliability: 2 Only 30 cells were analysed No positive control	Limited	Iso et al., 2006 ¹
Alkaline comet assay in CHO-K1 cell line <i>In vitro</i> chromosomal aberrations (Table 3)*	BPA 0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mM; 1 h exposure Positive control: H ₂ O ₂	BPA, purity > 99% (Tokyo Kasei Kogyo Co., Ltd)	Positive Increased DNA strand breaks	Reliability: 3 Non-standard method of DNA damage	Low	Tayama et al., 2008 ^{1*}

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	200 cells were scored Quantification of DNA damage: a score of 0–3 (mean score value = mean comet points/cell, comet points) Cell viability (trypan blue) Without metabolic activation		only at the highest concentration tested (0.7 mM)	quantification Data of cytotoxicity not clearly reported		
Alkaline comet assay HepG2 cells Cell viability: MTT test Bacterial reverse mutation assay (Table 1)*	BPA 0, 0.1, 1.0 and 10.0 µM for 4 and 24 h; 50 nuclei scored/treatment; at least 2 independent experiments; positive control: benzo[a]pyrene MTT test: 12.5, 25, 50, 100 µM for 24 h	BPA, purity >99% (Sigma-Aldrich)	Negative after 4 h of exposure Equivocal after 24 h exposure (no concentration-related effect) No cytotoxicity was observed	Reliability: 2 Only 50 nuclei scored	Limited	Fic et al., 2013 ^{1*}
Comet assay in rat INS-1 insulinoma cells Cell viability: Hoechst staining kit and trypan blue (apoptotic cells detection) Expression of nuclear p53 and p-Chk2 (T68) proteins: western blotting Intracellular (ROS): DCFH-DA	BPA 0, 25, 50, 100 µM for 24 h; or pre-treatment with or without NAC (10 mM) for 1 h then BPA (100 µM) was added for 24 h; Without metabolic activation 50 cells/slide were analysed; 3 experiments ROS and GSH analysis: BPA 0, 25, 50, 100 µM for 24 h	BPA, purity 99% (Sigma-Aldrich)	Positive concentration-related increase in tail DNA %, tail moment and tail length at 50 and 100 µM Significant decrease in tail DNA % in cells pre-treated with NAC No apoptotic cells and 90% cell survival were used in comet assays	Reliability: 2 No positive control; results on cytotoxicity assessment are not reported	Limited	Xin et al., 2014 [RefID 8147]

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Glutathione (GSH): detection with <i>o</i> -phthalaldehyde (OPT)	ROS measurements also in cells pre-treated with NAC and exposed to 100 µM BPA		(results are not shown) Increase of expression of DNA damage-associated proteins: p53 (from 50 µM) and p-Chk2 (at 100 µM) Levels of p53 are reduced by NAC pre-treatment Intracellular ROS: increase at 50 and 100 µM Decrease of ROS upon NAC pre-treatment GSH: concentration-related decrease			
Alkaline comet assay in CHO cells Cytotoxicity: MTT assay Bacterial reverse mutation assay (Table 1) <i>In vitro</i> chromosomal aberration (Table 3) micronucleus assays (Table 4)*	BPA 0, 40, 80, 100 and 120 µM for 12 and 24 h; 100 cells were analysed/sample. Without metabolic activation MTT assay: BPA 0, 40, 80, 100 and 120 µM for 12 and 24 h	BPA (purity 99%) ² , was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China)	Positive Concentration-related increase in (%) tail DNA from 80 µM with 12 h treatment, and at all tested concentrations after 24 h MTT assay: decrease in cell viability (but less than 50%) from 80	Reliability: 2 No positive control	Limited	Xin et al., 2015* [RefID 8150]

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			µM after 12 and 24 h			
Alkaline comet assay NIH3T3 cells (mouse embryonic fibroblast cell line) At least 100 nucleoids/sample Cytotoxicity: CCK-8 assay and LDH release Intracellular ROS: DCFH-DA 8-OHdG: EpiQuick 8-OHdG DNA damage quantification direct kit γH2AX: immunofluorescence and western blot	BPA 0, 2, 10 and 50 µM (0.4–11 µg/mL) for 24 h CCK-8 and LDH assays, ROS, 8-OHdG, γH2AX analysis: BPA 0, 2, 10 and 50 µM for 24 h At least 100 nucleoids of each sample were obtained in 3 independent experiments without metabolic activation	BPA (Sigma-Aldrich) purity >97% not reported in the study but available on the website of the company	Positive increase tail DNA% at 50 µM Cytotoxicity: 80% cell survival at 50 µM γH2AX, ROS and 8-OHdG: increase at 50 µM	Reliability: 2 No positive control	Limited	Chen et al., 2016 [RefID 1130]
Alkaline comet assay in FRTL-5 rat immortalised thyrocyte cell line Cell proliferation (population doubling) Transcriptome analysis (microarray) Intracellular ROS: H2DCFDA	BPA 10 ⁻⁹ M for 6h, 48h, 96 h; 100 cells for each condition Transcriptome analysis and intracellular ROS: cells exposed for 1, 3, and 7 days to 10 ⁻⁹ M BPA Without metabolic activation	BPA (Sigma-Aldrich), purity ≥97% not reported in the study but available on the website of the company	Comet assay on BPA alone: Negative Intracellular ROS: statistically significant increase after 1 and 3 days exposure Transcriptome analysis: decreased expression of genes involved in DNA replication, recombination and repair (confirmed by	Reliability: 3 Comet assay: - one low concentration tested; - no positive control Small effects on transcription Large variations in DNA strand breaks in the comet assay	Low	Porreca et al., 2016 [RefID 5892]

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			RT-PCR) (after 3 and 7 days BPA exposure) Cell proliferation: no effect			
Comet assay MCF-7 cells (from human breast adenocarcinoma) Cell viability: CCK-8 assay Cell membrane damage: LDH ROS formation: DCFH-DA	BPA 0, 1, 10, 25, 50 µM; 24 h Positive control: tBHP (<i>tert</i> -butyl hydroperoxide); 300 cells from each sample were analysed Without metabolic activation CCK-8 assay: 0, 0.01, 0.1, 1, 10, 25, 50, 100 µM for 24 h LDH: 0, 1, 10, 25, 50, 100 µM for 24 h ROS: 0, 0.01, 0.1, 1, 10, 25, 50 µM for 24 h	BPA, purity > 98% (Tokyo Chemical Industry)	Positive Concentration-dependent increase in % tail DNA from 10 µM Cell viability: at 1 µM increase in cell viability; inhibition of cell viability at concentrations from 10 µM (70%) to 100 µM (80%) Cell membrane damage: increase in LDH release in a concentration-dependent manner from 10 µM ROS formation: concentration-dependent increase in ROS levels No measurement at 50 µM, because of excessive cell death (90%)	Reliability: 3 Excessive toxicity at the analysed positive concentrations Results of positive control are not reported Comet methods are not described in detail	Low	Lei et al., 2017 [RefID 3960]

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Alkaline comet assay HepG2 cells Cytotoxicity: MTT assay Oxidative stress: intracellular ROS: DCFH-DA in the same cells, also MDA and SOD	BPA from 10 ⁻⁸ to 10 ⁻⁶ mol/L (0.02–22.8 µg/mL) for 24 h MTT: BPA from 10 ⁻⁸ to 10 ⁻⁴ mol/L for 24 h ROS, MDA and SOD analysis: BPA from 10 ⁻⁸ to 10 ⁻⁴ mol/L for 6 h Positive control: H ₂ O ₂	BPA purity > 99.8% (Sigma-Aldrich)	Positive Concentration-related increase of tail DNA (%) MTT: concentration-related increase of cytotoxicity; increase of ROS and MDA; decrease of SOD	Reliability: 2 No sufficient details on the comet method (e.g. number of cells analysed is not specified)	Limited	Li et al., 2017 [RefID 4176]
Alkaline and neutral comet assay Human PBMC (3 donors) 450 cells/concentration Cytotoxicity using flow cytometry	Alkaline comet assay: - BPA 0.1, 1 and 10 µg/mL for 1 h; - 0.01, 0.1, 1 and 10 µg/mL for 4 h Neutral comet assay: - BPA 0.1, 1 and 10 µg/mL for 1 h DNA repair: BPA at 10 µg/mL Without metabolic activation	BPA, 99–99.5% purity (Sigma-Aldrich)	Positive Both alkaline and neutral comet DNA repair of DNA breaks: decrease at 60 min, but the repair was not complete after 120 min	Reliability: 2 unusual software for comet analysis No positive control	Limited	Mokra et al., 2017 [RefID 5170]
Alkaline comet assay and modified comet assay with Fpg enzyme in human peripheral blood lymphocytes	1 h exposure to BPA: 0.001 mM, 0.1 mM, 2.5 mM Three experiments	BPA (Sigma-Aldrich) Purity ≥97% not reported in the study but available on the website of the company	Positive Increase of % tail DNA, only at the first 2 concentrations tested With Fpg a higher increase of % tail DNA was observed at all concentrations, but	Reliability: 3 Inadequate response of positive control; the use of hydrogen peroxide as positive control is not adequate for the comet + Fpg	Low	Durovcova et al., 2018 [RefID 262-G]

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			not concentration related	Number of cells scored in not specified		
Comet assay in human sperm cells Cell viability measured with a Nucleocounter NC 3000 <i>In vivo</i> comet assay (Table 8)**	BPA 0, 1, 1.5, 2 and 3 µmol/L for 1 h Without metabolic activation Each concentration was scored in 3 independent experiments and 2 replicates of each experiment 600 cells were scored/concentration Cell viability: BPA from 0 to 5 µmol/L	BPA (purity >99%, Sigma-Aldrich)	Negative No differences in % tail DNA between control samples and BPA-treated cells at all concentrations tested Cell viability assay: concentration-dependent decrease in cell viability from 3 µmol/L (reduced cell viability to 60%)	Reliability: 3 Test not validated and not adequate for cryopreserved samples	Low	Sharma et al., 2018** [RefID 662-G]
Comet assay in human bronchial epithelial BEAS-2B cells Cytotoxicity: MTS assay after 24 h treatment γ-H2AX foci using immunofluorescence Intracellular ROS: DCF proteins involved in the DNA damage response (p-ATM, p-ATR, p-Chk1, p-p53) using western blot	BEAS-2B cells were exposed to BPA 200 µM for 24 h MTS assay: 12.5 to 200 µM; tests performed in triplicates and for at least 3 independent times Without metabolic activation	BPA (Sigma-Aldrich) purity ≥97% not reported in the study but available on the website of the company	Increase of DNA damage, but no quantitative data are reported MTS assay: - concentration-dependent cytotoxic effect; - cytotoxicity at 200 µM: 84.7 ± 2.1%; γ-H2AX: BPA-induced phosphorylation BPA-induced also phosphorylation of ATM/ATR complex	Reliability: 3 Only one concentration tested, which resulted in high cytotoxicity Comet assay results not reported in detail, (no quantitative data) No positive control	Low	George and Rupasinghe, 2018 [RefID 277-G]

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			and triggered Chk1 and p53 proteins			
			Statistically significant increase of ROS			
Comet assay in TM3 murine Leydig cells Cell viability: MTT assay Real-time cell growth kinetics [cellular index (CI)] Cell-cycle analysis (PI, FACS analysis) Morphological analysis of cell death: chromatin staining with the Hoechst 33342 dye	BPA 0, 1, 10 and 100 µM for 3 h; cell viability analysed with trypan blue exclusion method; Positive control: doxorubicin; 250 nucleoids were analysed in each repetition (3 experiments) Without metabolic activation BPA concentrations for MTT assay and real-time cell growth kinetics: 0, 0.5, 1, 5, 10, 50, 100, 250, 500 µM MTT assay exposure: 24 or 48 h Real-time cell growth kinetics: measurement every 30 min for 96 h Cell-cycle analysis, chromatin staining: BPA 0, 1, 10 and 100 µM for 24 or 48 h	BPA (Sigma-Aldrich) purity ≥97% not reported in the study but available on the website of the company	Negative No increase in damage index (DI) Cell viability was evaluated using trypan blue exclusion method, and only treatments with an index greater than 80% were considered (results not shown) Cell viability: statistically significant and concentration-related decrease from 5 and from 50 µM after 24 and 48 h exposure, respectively CI: TM3 cells exhibited a decrease in their CI after 34 h of exposure at concentrations from 10 µM BPA 100, 250 and 500 µM decreased CI within a few hours of exposure	Reliability: 3 Results are reported as damage index (not a standard parameter)	Low	Gonçalves et al., 2018 [RefID 11104]

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			Cell-cycle analysis: BPA 100 µM induced an increase in the sub-G1 phase cell population No other effects induced in the distribution of TM3 cells in the G0 + G1, S, and G2 + M phases Morphological analysis of cell death: increase in chromatin staining upon exposure to BPA 100 µM for 24 or 48 h			
Alkaline comet assay with repair enzymes [with DNA glycosylases, i.e. endonuclease III (Nth) and human 8-oxoguanine DNA glycosylase (hOGG1)] Oxidised purines and pyrimidines Human PBMC 300 comets from 2 independent experiments Cell viability: flow cytometry	BPA 0, 0.01, 0.1 and 1 µg/mL for 4 h and 0, 0.001, 0.01 and 0.1 µg/mL for 48 h Positive control: H₂O₂ (2 blood donors) Without metabolic activation	BPA, 99–99.5% purity (Sigma-Aldrich)	Positive After 4 h incubation: - statistically significant and concentration-dependent oxidative damage to purines (from 0.01 µg/mL) and to pyrimidines (from 0.1 µg/mL) After 48 h incubation: - concentration-dependent oxidative DNA damage to purines (from 0.001 µg/mL) and to	Reliability: 2 No appropriate positive control unusual software for comet analysis	Limited	Mokra et al., 2018 [RefID 364-G]

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			pyrimidines from (0.01 µg/mL) Statistically significant differences for DNA damage between 4 h and 48 h exposure at the highest concentrations tested (0.01 and 0.1 µg/mL) Cell viability: no significant changes			
Alkaline comet assay (CometChip platform) in mouse embryonic fibroblasts (MEF) Analysis of γH2AX (immunofluorescence) <i>In vitro</i> chromosomal aberrations test (Table 3)*	BPA 150 µM for 24 and 48 h (24 h for γH2AX), or co-exposure with camptothecin (CPT) Data of 4 replicates, each with 1500 ± 300 comets Without metabolic activation	BPA (Sigma-Aldrich) purity ≥97% not reported in the study but available on the website of the company	Negative No significant increase in the % tail DNA No significant increase in the percentage of γH2AX-positive nuclei	Reliability: 3 No positive controls, no sufficient details on the methods applied; single concentration; cytotoxicity not evaluated	Low	Sonavane et al., 2018* [RefID 419-G]
Comet assay in murine macrophage RAW264.7 cells Cell viability: MTT assay Intracellular ROS level: semiquantitative DCFH-DA fluorescence assay Assessment of the antioxidative enzymes activities: CAT, SOD, and GPx	BPA 0, 3, 10, 30, or 50 µM for 24 h; no positive control; a minimum of 50 cells/slide were analysed MTT assay: BPA 0, 3, 10, 30, or 50 µM for 12 or 24 h DCFH-DA assay and assessment of antioxidative enzymes activities:	BPA (Sigma-Aldrich) purity ≥97% not reported in the study but available on the website of the company	Positive Increase in tail moment and tail length in a concentration-dependent manner starting from 10 µM of BPA Cytotoxicity: concentration- and time-dependent	Reliability: 2 No positive control	Limited	Huang et al., 2018* [RefID 296-G]

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<i>In vitro</i> micronucleus assay (Table 4)*	- BPA 0, 3, 10, 30, or 50 µM for 24 h Without metabolic activation		decrease of cell viability BPA-induced ROS generation and reduced antioxidative enzyme activities from 10 µM			
Comet assay and comet modified with FpG In cryopreserved: - Hep-2 cells (human epithelial cells from laryngeal carcinoma); - MRC-5 cells (DNA damage responsive cell line, human lung fibroblasts) Cell viability: CellTiter-Blue assay <i>In vitro</i> micronucleus assay (Table 4)*	BPA 0.44 nM, 4.4 nM, 4.4 µM for 48 h; Hep-2 cells: 300 cells analysed for each treatment MRC-5 cells: 100 cells analysed for each treatment Cell viability: BPA 0.44 nM, 4.4 nM, 4.4 µM, 48 h exposure in both Hep-2 and MRC-5 cells	BPA (Sigma) purity ≥97% not reported in the study but available on the website of the company	Inconclusive	Reliability: 3 Comet assay is not validated and recommended for testing cryopreserved cell samples No positive control	Low	Ramos et al., 2019* [RefID 396-G]
Comet assay in sperm cells from Sprague Dawley rats Analysis: ROS, LPO, SOD <i>In vivo</i> comet assay (Table 8)**	BPA 0, 1, 10, and 100 µg/L for 2 h No positive control Without metabolic activation	BPA (99% purity) Santa Cruz Biotechnology	Positive Increase of tail DNA% only at 100 µg/L BPA increased SOD, ROS, TBARS [thiobarbituric acid reactive substances (TBARS) as an index of LPO] only at 100 µg/L	Reliability: 3 The study was performed following a non-standard, neutral protocol and unusual evaluation of comets based on the analysis of microphotographs. No positive control	Low	Ullah et al., 2019** [RefID 443-G]

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Comet assay in Marc-145 cells (rhesus monkey embryo renal epithelial cells) Cytotoxicity: MTT and LDH assays Intracellular ROS levels: DCFH-DA Lipid peroxidation: - TBARS; - SOD activity and GSH content	BPA 10^{-6} to 10^{-3} M for 24 h; 50 cells from each of 6 independent experiments were analysed MTT assay: BPA 10^{-6} to 10^{-1} M for 24 h; DCFH-DA and TBARS assays: BPA 10^{-6} to 10^{-3} M for 24 h; SOD activity and GSH content: BPA 10^{-6} to 10^{-3} M for 24 h Without metabolic activation	BPA (purity > 99%) Sigma-Aldrich	Positive Increase in % tail DNA, tail length and tail moment (10^{-6} - 10^{-3} M); Cytotoxicity: concentration-related increase; excess of toxicity at 10^{-3} and 10^{-4} M BPA DCFH-DA, TBARS assays: - concentration-related increase of ROS and lipid peroxidation; - SOD activity and GSH content: concentration-related decrease	Reliability: 2 No positive control	Limited	Yuan et al., 2019 [RefID 478-G]
Alkaline comet assay and Fpg modified comet assay RWPE-1 cells [human papilloma virus 18 (HPV18) immortalised, non-tumorigenic prostatic cell line] Cell viability: modified MTT assay and trypan blue exclusion Enzymatic and non-enzymatic antioxidants:	BPA 0, 45 μM (IC_{20}) for 24 h 450 comets analysed/treatment; experiments in triplicates Cell viability: 0, 50, 100, 200, 300, 600 μM for 24 h Enzymatic and non-enzymatic antioxidants: BPA 0, 45 μM (IC_{20}) for 24 h	BPA (>99% pure)	Positive Comet assay: increase (2.5-fold) in tail intensity (at IC_{20} BPA) Fpg modified comet: increase in tail intensity Cell viability: decrease in cell viability (IC_{20} 45 μM)	Reliability: 2 One concentration tested No positive control No metabolic activation	Limited	Kose et al., 2020 [RefID 325-G]

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analysis of GPx, GR, SOD, GSH and TAOC levels	Without metabolic activation		Enzymatic and non-enzymatic antioxidants: decrease in: - GPx1 and SOD activity (29% and 24% respectively); - TAOC levels (20%); increase in: - GR activity (4.5-fold); - total GSH level (30%)			
Comet assay in HepG2 cells (human hepatocellular carcinoma cell line) Cell viability: MTT test SOS/umuC assay (Table 1)*	BPA 0, 1, 10, 100 and 1000 µg/L, for 4 and 24 h; 3 independent experiments; 50 nuclei analysed/treatment MTT test: BPA 0, 1, 10, 100 and 1000 µg/L, for 24 h	BPA (Sigma-Aldrich) purity >97% not reported in the study but available on the website of the company	Positive increase of % tail DNA from 10 µg/L at both 4 h and 24 h exposure MTT test: no effects on cell viability	Reliability: 2 Low number of nuclei analysed	Limited	Balabanič et al., 2021* [RefID 224-G]

Table 6: *In vivo* chromosomal aberrations assay
(OECD TG 475 was considered for the evaluation of the reliability)

Test system/Test object	Dose/Route	Information on the characteristics of the test substance	Results	Reliability/Comments	Relevance of the result	Reference (authors and RefID)
Chromosomal aberration assay in bone marrow Swiss albino mice Six animals (3 females and 3 males)/group (control and BPA-treated animals) 100 metaphases were scored per animal Mitotic effects <i>In vivo</i> micronucleus assay (Table 7)*	BPA 0, 10, 50 and 100 mg/kg bw; 2% gum acacia was used as the suspending medium for BPA Single oral dose administered by gavage Sampling of bone marrow at 6, 24, 48 and 72 h Cumulative dose level: 10 mg/kg bw for 5 consecutive days Sampling of the bone marrow 24 h after the last administration of BPA	BPA, purity 98% (Loba Chemie, Mumbai, India)	Negative No significant increase of structural chromosomal aberrations Significant increases in the frequencies of gaps at all doses at 48 and 72 h sampling time and at 50 and 100 mg/kg bw at the 24 h sampling time C-mitotic effects through increases of mitotic indices and decrease in anaphase for both higher dose level at 24, 48 and 72 h sampling times	Reliability: 2 Low number of animals/sex, but in total 6 animals/group Low number of metaphases scored, treatment with colchicine shorter (1.5 h) than recommended (5–6 h)	Limited	Naik and Vijayalaxmi, 2009^{1*}
Chromosomal aberration in bone marrow Holtzman rats Ten animals (5 females and 5 males)/group (control and BPA-treated animals)	BPA 0, 2.4 µg, 10 µg, 5 mg and 50 mg/kg bw administered orally once a day for 6 consecutive days; BPA dissolved in distilled ethyl alcohol and diluted with sesame oil Sampling of the bone marrow 24 h after the	BPA, ~99% purity (Sigma Chemical Company)	Positive Dose-related increase of structural chromosomal aberrations starting from 10 µg	Reliability: 2 Mitotic index as a measure of cytotoxicity not determined	Limited	Tiwari et al., 2012^{1*,**}

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Analysis of 100 metaphases per animal	last administration of BPA					
<i>In vivo</i> micronucleus assay (Table 7)* and comet assay (Table 8)*						
Bacterial reverse mutation assay (Table 1)**						

Table 7: *In vivo* micronucleus assay
(OECD TG 474 was considered for the evaluation of the reliability)

Test system/Test object	Dose/Route	Information on the characteristics of the test substance	Results	Reliability/Comments	Relevance of the result	Reference (authors and RefID)
Micronucleus assay Male ICR mice Peripheral blood reticulocytes (1000/animal analysed, 5 mice per group) Bacterial reverse mutation assay (Table 1)**	228 mg/kg bw of BPA dissolved in DMSO, once by gavage; controls received vehicle alone Peripheral blood collected at 24, 48 and 72 h after administration	BPA purity >99% (Tokyo Kasei Kogyo Co., Ltd)	Inconclusive (negative with no demonstration of bone marrow exposure) No increase of micronucleated reticulocytes at any sampling time Cytotoxicity was not evaluated	Reliability: 2 Single dose tested, although relatively high; 1000 scored reticulocytes/animal instead of 2000 as in OECD TG 474 (1997) No positive control	Low	Masuda et al., 2005^{1**}
Micronucleus assay in bone marrow Male mice (102/ElxC3H/EI)F1 (5 animals per group)	BPA 0, 0.002, 0.02 and 0.2 mg/kg bw oral gavage on 2 days Cells collected 24 h after last administration 2000 polychromatic erythrocytes (PCE) were scored per animal	BPA (Sigma-Aldrich) purity >97% not reported in the study but available on the website of the company	Inconclusive (negative with no demonstration of bone marrow exposure) No induction of micronuclei in the bone marrow polychromatic erythrocytes	Reliability: 2 No positive control; very low doses applied	Low	Pacchierotti et al., 2008¹
Cytogenetic analyses of oocytes and zygotes in female C57Bl/6 mice Assessment of meiotic delay in spermatocytes by BrdU incorporation and aneuploidy in	Acute exposure: 0.2 or 20 mg/kg Sub-acute exposure: 0.04 mg/kg for 7 days by gavage Sub-chronic exposure: 0.5 mg/L for 7 weeks in drinking water	BPA (Sigma-Aldrich)	Negative No significant induction of hyperploidy or polyploidy in oocytes and zygotes in any treatment condition No delay of meiotic divisions	Reliability: 2 This study was adequately planned, performed and reported, even though specific guidelines for the effects in germ cells are not available	Limited	

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epididymal sperm by multicolor FISH in male 102/ElxC3H/El)F1 mice (5 mice per dose)	0.2 mg/kg bw starting on day 8 after BrdU, for 6 consecutive days BPA 0, 0.002, 0.02 and 0.2 mg/kg for 6 consecutive days		No induction of hyperploidy or polyploidy in epididymal sperms	No positive control Very low doses for the analysis of sperm aneuploidy		
Micronucleus in bone marrow Swiss albino mice Six animals (3 females and 3 males)/group (control and BPA-treated animals); 2000 PCE/animal <i>In vivo</i> chromosomal aberration (Table 6)*	BPA 0, 10, 50 and 100 mg/kg bw; 2% gum acacia was used as the suspending medium for BPA Single oral dose administered by gavage sampling of bone marrow at 6, 24, 48 and 72 h Cumulative dose level: 10 mg/kg bw for 5 consecutive days Sampling of the bone marrow 24 h after the last administration of BPA	BPA purity 98% (Loba Chemie, Mumbai, India)	Negative No significant decrease of PCE/NCE ratio Significant increase of gaps and C-mitoses	Reliability: 2 Low number of animals/sex in each group, but in total 6 animals/group	Limited	Naik and Vijayalaxmi, 2009 ^{1*}
Micronucleus in bone marrow Male Sprague Dawley rats 8 rats/group (control and BPA-treated animals) <i>In vivo</i> comet assay (Table 8)*	BPA 0, 200 mg/kg bw per day for 10 days Orally via drinking water Bone marrow processed at the end of treatment	BPA (Sigma-Aldrich) purity >97% not reported in the study but available on the website of the company	Inconclusive (negative with no demonstration of bone marrow exposure) No data on bone marrow toxicity are reported	Reliability: 2 Exposure of the bone marrow not demonstrated Single dose tested No positive control	Low	De Flora et al., 2011 ^{1*}

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Micronucleus in bone marrow Holtzman rats Ten animals (5 females and 5 males)/group (control and BPA-treated animals) <i>In vivo</i> chromosomal aberration (Table 6)* Comet assay (Table 8)* Bacterial reverse mutation assay (Table 1)**	BPA 0, 2.4 µg, 10 µg, 5 mg and 50 mg/kg bw per day administered orally for 6 consecutive days Sampling of the bone marrow 24 h after the last administration of BPA Analysis of 2000 PCE	BPA, ~99% purity (Sigma Chemical Company)	Positive Dose-related increase of MN-PCE starting from 10 µg/kg bw per day	Reliability: 2 Inappropriate staining	Limited	Tiwari et al., 2012^{1*},**
Micronucleus test in peripheral blood reticulocytes and in bone marrow of Pzh:Sfis female mice No. of animals/group: 9 in control, 6 in BPA 5 mg/kg bw, 8 in BPA 10 mg/kg bw, 6 in BPA 20 mg/kg bw; 1000 reticulocytes or PCE were scored <i>In vivo</i> comet assay (Table 8)*	BPA 5, 10, or 20 mg/kg bw per day for 2 weeks in drinking water Animals were sacrificed 24 h after the end of treatment Blood was collected at 1 and 2 weeks of exposure	BPA, no information on purity or the supplier company	Positive in reticulocytes at 10 and 20 mg/kg bw after 2 weeks of exposure Negative in reticulocytes after 1 week of treatment Negative in bone marrow	Reliability: 2 No criteria for scoring micronuclei were described No positive control	Low No information on source and purity of BPA	Gajowik et al., 2013* [RefID 2120]

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Micronucleus test in bone marrow cells Adult male Wistar albino rats Ten animals per group	Oral administration of 5 µg, 50 µg and 100 µg BPA/100 g bw once a day for 90 days, sacrifice and sampling of bone marrow on the 91th day	BPA (<99% pure) purchased from Sigma-Aldrich, diluted in olive oil	Positive Increases (2–3-fold at the highest dose) in the frequency of micronuclei in polychromatic erythrocytes and normochromatic erythrocytes Statistical significance of the difference with negative controls not determined No decrease in PCE/NCE ratio	Reliability: 3 Major limitation in data presentation and analysis: low number of scored cells per animal lack of historical control data	Low	Srivastava and Gupta, 2016 [RefID 9611]
Micronucleus test in bone marrow Male Swiss albino mice, 10 animals/group; analysis of 2000 PCE/animal <i>In vivo</i> comet assay (Table 8)*	50 mg/kg bw, orally once a day for 28 days Sampling of the bone marrow at the end of treatment	BPA, purity ≥ 99%, (Sigma-Aldrich)	Positive Increase in the mean values of MNPCEs (66.40 ± 9.94 vs 10.40 ± 2.96) Cytotoxic (reduction in the ratio of PCE/NCE compared to control)	Reliability: 2 No positive control only one dose	Limited	Fawzy et al., 2018* [RefID 270-G]
Micronucleus test in bone marrow Male Wistar rats; 6 animals/group Analysis of 2000 PCE for MN scoring and of 200 cells for PCE/NCE ratio	0, 50 and 100 µg/kg bw per day, 4 weeks, by gavage Sampling at the end of treatment	BPA (Sigma-Aldrich) purity >97% not reported in the study but available on the website of the company	Positive Significant dose-related increase (up to 3-fold) in the mean values of MNPCEs compared with control Cytotoxic (a weak statistically significant decrease in PCE/NCE	Reliability: 2 No positive control only 2 doses	Limited	Panpatil et al., 2020* [RefID 379-G]

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Lipid peroxidation: serum level of malondialdehyde (MDA) (8-OHdG) in urine <i>In vivo</i> comet assay (Table 8)*			ratio); dose-related increase of MDA in blood and of urinary 8-OHdG levels			
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Table 8: *In vivo* DNA damage

(comet assay, OECD TG 489 was considered for the evaluation of the reliability)

Test system/Test object	Dose/Route	Information on the characteristics of the test substance	Results	Reliability/ Comments	Relevance of the result	Reference (authors and RefID)
Comet assay in peripheral blood lymphocytes Sprague Dawley rats 8 rats/group (control and BPA-treated animals); 100 nuclei were scored <i>In vivo</i> micronucleus assay (Table 7)*	200 mg/kg bw for 10 consecutive days, orally via drinking water Sampling at the end of treatment	BPA (Sigma-Aldrich) purity >97% not reported in the study but available on the website of the company	Negative	Reliability: 2 Tail moment, used as only parameter to report the results for the comet assay, is not recommended by the Comet international Committee; single dose tested; no positive control	Limited	De Flora et al., 2011 ^{1*}
Comet assay in peripheral whole blood cells of Wistar rats (6 animals/group BPA-treated animals; 5 animals in the control group; 3 animals in the positive control group)	0, 125 and 250 mg/kg bw; oral administration (gavage) for 4 weeks Positive control: MMS (i.p., sampling after 24 h); 50 cells were analysed on each replicated slide	BPA purity > 99% (Merkolab Chemistry)	Positive Increase of both tail length and tail moment at 250 mg/kg bw	Reliability: 3 Inappropriate presentation and evaluation of results Group mean tail length and tail moment values, rather than the means of animal median values (OECD TG 489) Sampling time, and frequency of administrations not stated	Low	Ulutaş et al., 2011 ¹

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Comet assay in blood lymphocytes Holtzman rats Ten animals (5 females and 5 males)/group (control and BPA-treated animals); analysis of 50 nucleoids/animal Plasma concentrations of 8-hydroxydeoxyguanosine (8-OHdG), lipid peroxidation (MDA) and glutathione activity <i>In vivo</i> micronucleus assay (Table 7)* chromosomal aberrations assay (Table 6)* Bacterial reverse mutation assay (Table 1)**	2.4 µg, 10 µg, 5 mg and 50 mg/kg bw per day administered once a day for 6 consecutive days Sampling 24 h after the last administration of BPA	BPA, ~99% purity (Sigma Chemical Co.)	Positive Dose-related increase starting from 10 µg/kg bw per day Significant increase in plasma concentration of 8-OHdG only at 50 mg/kg bw per day Dose-related increase of MDA and decrease of glutathione in liver Inconsistent results of 8-OHdG with comet assay	Reliability: 2 Inappropriate sampling time Low number of nucleoids scored	Limited	Tiwari et al., 2012^{1*}, ^{**}
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Comet assay in bone marrow, spleen, liver and kidney and germ cells Male Pzh:SFIS mice; 5 animals/group; 100 cells were analysed	0, 5, 10, 20 or 40 mg/kg bw Orally in drinking water Daily for 2 weeks Animals were sacrificed 24 h after the last treatment	BPA, no information on purity or the supplier company	Positive Increases of DNA tail moment in bone marrow, spleen, kidney and lung cells at any dose level without a clear dose-response No increase of tail moment was detected in liver cells In sperm cells increase of tail moment: at all doses 24 h after the end of exposure; at the 2 highest doses 5 weeks after the end of treatment	Reliability: 3 No information on purity; drinking water consumption (containing BPA) not measured, inadequate sampling time, poor study report; tail moment, used as only parameter to report the results for the comet assay, is not recommended by the Comet International Committees	Low	Dobrzyńska and Radzikowska, 2013 ¹
Alkaline comet assay in epididymal sperm of Holtzman rats <i>In vivo</i> dominant lethal mutations in male rats (Table 9)*	Oral gavage of 10 µg/kg bw and 5 mg/kg bw BPA dissolved in ethyl alcohol and diluted in sesame oil, for 6 consecutive days	BPA ~99% purity (Sigma Chemical Co.)	Positive Significant increase in the sperm DNA damage at 5 mg/kg bw	Reliability: 3 Comet assay is not considered appropriate to measure DNA strand breaks in mature germ cells due to the high and variable background levels in DNA damage in this cell type (OECD TG 489); moreover, the sampling time, i.e. 8 weeks after last treatment, is inappropriate for <i>in vivo</i> comet assay	Low	Tiwari and Vanage, 2013 ^{1*}

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Comet assay in lung, spleen, kidneys, liver and bone marrow of Pzh:Sfis female mice No. of animals/group 9 in control, 6 in BPA 5 mg/kg bw, 8 in BPA 10 mg/kg bw; 6 in BPA 20 mg/kg bw 100 nucleoids scored/animal <i>In vivo</i> micronucleus assay (Table 7)*	BPA 5, 10, or 20 mg/kg bw/day for 2 weeks in drinking water Sampling 24 h after the end of treatment	BPA, no information on purity or the supplier company	Positive in lung at 5 and 10 mg/kg Negative in spleen, kidneys, liver and bone marrow	Reliability: 2 Inappropriate sampling time, tail moment, used as only parameter to report the results for the comet assay, is not recommended by the Comet International Committees	Low No information on source and purity of BPA	Gajowik et al., 2013* [RefID 2120]
Alkaline comet assay in brain cells of KM male mice; (11 animals/group); 200 cells for each group analysed	BPA 0.5, 50 and 5000 µg/kg bw (daily dose, diluted in tea oil, by gavage) for 8 weeks After 8 weeks of exposure, mice were sacrificed and the brain samples were immediately removed The tail DNA%, tail length and tail moment were measured using CASP comet analysis software Based on the DNA percentage of the tail intensity, the damage level was divided into 5 grades	BPA from Sigma-Aldrich (HPLC grade) purity >97% not reported in the study but available on the website of the company	Positive Significant increase of damaged cells from 23.0% in the control group to 47.3%, 66.6% and 72.5% in the low-, medium and high-exposed groups Severity of DNA damage, expressed as arbitrary units (AUs), increased with AUs of 0.28 in the control to AUs of 0.59, 0.96 and 1.28 in the low, medium and highly exposed groups, respectively	Reliability: 2 DNA damage was evaluated using arbitrary units and considering the distribution of DNA damage in the cell population analysed (n = 440), rather than using median animals data as the statistical unit, as recommended in OECD TG 489	Limited	Zhou et al., 2017 [RefID 9083]

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	Arbitrary units computed with the score of DNA damage in analysed cells were used to express the DNA damage					
Comet assay in liver female Wistar rats; (7 animals/group) Serum biochemical analysis: ALT, ALP, TP, Alb, GGT, TC, Triglycerides, HDL; LDL Hepatic antioxidants and lipid peroxidation level: GPx, SOD, MDA CYP450 (ELISA) Histopathology Immunohistochemical evaluation of caspase-3	7 animals/group: control (corn oil) BPA 10 mg/kg bw; daily administration via gavage for 30 days Sampling at the end of treatment	BPA (Sigma-Aldrich) purity >97% not reported in the study but available on the website of the company	Positive: increase of tail DNA % BPA-induced: - increase of ALT, ALP, GGT, TC, LDL, MDA, caspase-3; - decrease of Alb, TP, GPx, SOD, CCYPR450 Histopathological analyses showed deleterious hepatic changes ranging from hepatocytes' vacuolisation and eccentric nuclei to focal necrosis and fibrosis	Reliability: 3 Use of frozen tissues; without a positive control; a single dose applied; toxic effects in liver	Low	Abdel-Rahman et al., 2018 [RefID 199-G]
Comet assay in liver of Sprague Dawley rats of either sex; 7 animals/group Serum analysis: ALT, ALP, AST, bilirubin Analysis of antioxidant effects: CAT, POD, SOD, GSH Lipid peroxidation assay, hydrogen peroxide assay, nitrite assay	BPA, 25 mg/kg by i.p. negative control group; vehicle control group (10% DMSO in olive oil) Sampling: 4 weeks after the treatment	BPA, no information on purity or the supplier company	Positive increase of tail DNA % 28.35 ± 1.2 vs 0.01 ± 0.005 BPA-induced: - increase of WBC, ALT, AST, ALP, bilirubin, H_2O_2 , nitrite - decrease of RBC, platelets, Hb, albumin, CAT, POD, SOD, GSH, 'Histopathological examination of BPA-	Reliability: 3 Limitations: - a single administration by i.p. and comet, analysis after 4 weeks; - unusual software used for the comet analysis; - the results reported using the different parameters (tail length, % of DNA in tail, tail moment) are not consistent;	Low A single administration by i.p. No information on source and purity of BPA	Kazmi et al., 2018 [RefID 315-G]

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Liver histopathology			treated animals revealed intense hepatic cytoplasm inflammation, centrilobular necrosis, cellular hypertrophy, fatty degeneration, vacuolisation, steatosis and distortion of portal vein'	- the value of % of DNA in tail in controls is extremely low with respect to the data reported in the scientific literature; - high liver toxicity		
Comet assay in liver of Male Swiss albino mice (10 animals/group); images of 50 randomly selected nuclei/ experimental group Analysis of liver toxicity markers (AST and ALT) and liver histopathology	BPA dissolved in ethanol and diluted in corn oil by gavage at 50 mg/kg bw, once a day for 28 successive days	BPA ($\geq 99\%$) Sigma-Aldrich	Positive Mean tail length, tail moment and % tail DNA were significantly increased ($p < 0.05$) in liver of BPA-treated mice Increase of AST, ALT, marked histopathological alteration in liver of BPA-treated animals 'congestion of the hepatic blood vessels as well as marked vacuolar degeneration of the hepatocytes with many necrotic cells'	Reliability: 3 Major deviation from OECD TG 489: -too low number of analysed cells per animal -aggregated mean data analysed (instead of animal median) -no positive control - too high liver toxicity associated with treatment	Low	Elhamalawy et al., 2018 [RefID 510-G]
Alkaline comet assay in liver, kidney, testes, urinary bladder, colon and lungs cells CD-1 male mice (5 mice/group) <i>In vitro</i> comet assay (Table 5)**	Gavage 0, 125, 250 and 500 mg/kg bw BPA (maximum tolerated dose) as suspensions in corn oil prepared by ultrasonication 2 doses (24 h apart) Animals were sacrificed 3 h after 2nd dose	BPA (purity $>99\%$, Sigma-Aldrich)	Negative None of the tissues showed an effect of BPA except in testicular cells, in which an increased level of DNA strand breaks ($p < 0.01$ compared with control group) was observed at the lowest dose only	Reliability: 1 This study basically followed the OECD TG 489	High	Sharma et al., 2018** [RefID 662-G]

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	200 cells analysed/mice (100 cells per gel and 2 gels per mouse)					
Comet assay in liver and testes of male Swiss albino mice Male Swiss albino mice, 10 animals/group 50 nuclei/group were analysed <i>In vivo</i> micronucleus assay (Table 7)*	50 mg/kg bw, orally once a day for 28 days Sampling at the end of treatment	BPA, purity $\geq$ 99% (Sigma-Aldrich)	Positive Increase ($p \leq 0.05$) in the mean values of tail length, percentage of tail DNA and Olive tail moment in liver and testes Histopathological examination hepatocyte vacuolar degeneration with many necrotic cells Defective spermatogenesis characterised by severe necrosis and loss of the spermatogonial layers with multiple spermatid giant cells formation in most of the seminiferous tubules and a congestion of the interstitial blood vessels	Reliability: 3 No positive control, low number of nucleoids analysed, toxic effects observed in liver and testes, a single dose applied The standard alkaline comet assay applied is not considered appropriate to measure DNA strand breaks in mature germ cells	Low	Fawzy et al., 2018* [RefID 270-G]
Comet assay in heart of Wistar rats; 20 animals/group	BPA dissolved in corn oil 30 mg/kg bw per day injected subcutaneously (SC) 6 days/week for 4 weeks Sacrifice at the end of treatment	BPA Sigma-Aldrich; purity >97% not reported in the study but available on the website of the company	Positive Increase tail DNA % (6.88 vs 1.67) Histopathological changes: focal disruption of cardiomyocytes with some nuclear changes, such as karyolysis and pyknosis and sarcoplasmic vacuolisation The mitochondria appeared swollen and	Reliability: 3 Single dose; no positive control; inadequate cell preparation for comet assay; high toxicity	Low route of administration: subcutaneous	Amin et al., 2019 [RefID 216-G]

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			deranged with different sizes and shapes			
Comet assay in testes of Sprague Dawley rats; 7 rats/group Histopathology Antioxidant enzymes: CAT, SOD, GSH, POD, NO	BPA (50 mg/kg bw) injected intraperitoneal on alternate days for 21 days Sacrifice 24 h after the end of treatment	BPA analytical grade (Merck KGaA); purity >97% not reported in the study but available on the website of the company	Positive Histopathology: 'BPA caused significant damage and abrasions to seminiferous tubules with low cellular density' BPA-induced: - decrease of body weight, epididymis and testes weight, testosterone, FSH, LH, CAT, SOD, GSH, POD; - decrease of sperm count, viability, motility - increase of estradiol	Reliability: 3 Single dose; no positive controls; an unusual software for the comet analysis used; the comet presented in the microphotographs are of low quality The standard alkaline comet assay applied is not considered appropriate to measure DNA strand breaks in mature germ cells	Low BPA was administered by i.p.	Majid et al., 2019 [RefID 354-G]
Comet assay (neutral) on spermatozoa of Sprague Dawley rats (7 per group) 100 scored cells per animal <i>In vitro</i> comet assay (Table 5)**	Animals treated by gavage with 5, 25 and 50 mg BPA/kg bw per day for 28 days and sacrificed on day 29 th , control received the vehicle alone (0.1% ethanol)	BPA (99% purity) from Santa Cruz Biotechnology	Positive Both tail moment and % tail DNA were significantly ($p < 0.05$) increased in the BPA 50 mg/kg bw per day group compared to vehicle controls, while no significant difference with controls was observed in the BPA 5 and 25 mg/kg bw per day groups	Reliability: 3 The study was performed following a non-standard, neutral protocol and unusual evaluation of comets based on the analysis of microphotographs No detailed information on data analysis is provided (e.g. the use of median vs mean as individual animal descriptor) No positive control	Low	Ullah et al., 2019** [RefID 443-G]

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Comet assay in testes of offspring of BPA-treated mice (pregnant Kummung mice, 20 in each group)	Animals were randomly divided into 7 groups. One group served as control, the others received BPA in drinking water at 0.05, 0.5, 5, 10, 20 or 50 mg/kg bw per day, for 40 days from gestation day 0 to lactation day 21. F1 male mice were sacrificed at weaning (post-natal day 21) and DNA damage in testes evaluated by comet assay	BPA (purity 99%, Sigma)	Positive The results obtained showed significantly increased Olive tail moment (OTM) in testes cells of F1 animals treated with 5, 10, 20 and 50 mg/kg bw per day, compared with the control group ($p < 0.05$).	Reliability: 3 The standard alkaline comet assay applied (OECD TG 489) is not considered appropriate to measure DNA strand breaks in mature germ cells. Moreover, no detailed experimental data and information on test method are presented. OTM is not recommended as the unique parameter to evaluate DNA damage in comet assay; a positive control was not performed	Low	Zhang et al., 2019 [RefID 486-G]
Alkaline comet assay in thyroid tissue Male albino rats 20 rats/group Biochemical investigation of MPO activity, GSH, SOD activity and MDA	BPA dissolved in corn oil 200 mg/kg bw per day (1/20 of the oral LD ₅₀) for 35 days Sacrifice 24 h after the last administration	BPA (99.5% purity) was obtained from Sigma-Aldrich Co.	Positive % tail DNA 4 times increase compared with control level The histopathological examinations of thyroid gland showed severe congestion of interstitial blood capillaries, severe lymphocytic infiltration associated with variable-sized follicles, most of which contain scanty colloid secretion, and	Reliability: 3 Only one dose level No positive control Comet method poorly described The microphotographs of comets are of low quality High toxicity	Low	Mohammed et al., 2020 [RefID 363-G]

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			some are atrophied in BPA group Significant induction of MPO activity and MDA concentration associated with significant decreases of SOD activity and GSH concentration in the thyroid gland of BPA group			
Alkaline comet assay in testes Male juvenile Sprague Dawley (SD) rats (7 animals/group) Sperm DNA damage was evaluated by the comet and Halo assays using duplicate slides; apoptosis in testes cells was quantified using TUNEL assay, and testicular levels of 8-OHdG were determined by immunohistochemistry	Gavage 8 weeks BPA (100 mg/kg bw per day) daily/5 days per week by gavage for 8 consecutive weeks Animals were sacrificed after 8 weeks	BPA (Sigma-Aldrich) Purity >99% not reported in the study but available on the website of the company	Negative All comet assay parameters (tail length, Olive tail moment and % DNA in the tail) and the nuclear diffusion factor in Halo assay, were slightly but not significantly increased in testes cells of BPA-treated rats compared with controls TUNEL-positive cells and per cent of 8-OHdG positive areas in testicular tissue were also slightly but non-significantly increased in BPA-treated rats	Reliability: 3 The standard alkaline comet assay applied (OECD TG 489) is not considered appropriate to measure DNA strand breaks in mature germ cells Other test methods (Halo and immunohistochemical determination of 8-OHdG) are not standardised and/or validated for regulatory use For all end-points, only a single dose was tested Sampling time not specified No positive control	Low	Sahu et al., 2020 [RefID 405-G]
Comet assay on whole brain cells from KM	Pregnant mice (F0) were orally dosed with BPA	BPA (purity: 98 %) Sigma-Aldrich	Equivocal	Reliability: 3	Low	Zhang et al., 2020 [RefID 485-G]

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mice of F1 and F2 (8 male and 8 female)	dissolved in tea oil at 0.5, 50, 5000 µg/kg bw per day from gestational day 1 until weaning (post-natal day 21). Then, the first generation (F1) of mice were used to generate the F2 DNA damage in brain cells was evaluated by comet assay in mice from both F1 and F2		DNA damage, expressed as arbitrary units, was slightly (less than two-fold) increased in the F1 male mice at the lowest dose and in females at the intermediate dose. No effect of BPA exposure was observed in the F2 mice	The study protocol is only shortly described The presentation and interpretation of the results is inadequate No positive control		
Comet assay in blood liver and kidney Male Wistar rats (WNIN) 6 animals/group 50 nuclei/slides were scored Lipid peroxidation: serum level of malondialdehyde (MDA) 8-Hydroxy-2'-deoxyguanosine (8-OHdG) in urine collected 24 h before the sacrifice <i>In vivo</i> micronucleus assay (Table 7)*	0, 50, and 100 µg/kg, per oral (gavage) for a period of 4 weeks Sampling at the end of treatment	BPA, (Sigma-Aldrich) purity >97% not reported in the study but available on the website of the company	Positive A weak but statistically significant and dose-related increase of tail length in liver In kidney increase of DNA damage observed only at the dose of 50 µg/kg Comet parameters are not reported for blood cells Dose-related increase of MDA in serum and of 8-OHdG levels in urine	Reliability: 2 Low number of nucleoids analysed No positive controls	Limited	Panpatil et al., 2020* [RefID 379-G]
Evaluation of sperm DNA damage by alkaline comet and DNA ladder assays	BPA diluted in 10% DMSO was injected intraperitoneally at 25 mg/kg bw on alternate days for 30 days	BPA, no information on purity or the supplier company	Positive Significant ($p < 0.01$) increase of all comet parameters in BPA-treated	Reliability: 3 The standard alkaline comet assay applied (OECD TG 489) is	Low For insufficient reliability and lack	Zahra et al., 2020 [RefID 481-G]

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Male Sprague Dawley rats (groups of 7 animals)			animals compared with vehicle controls Electrophoresis on agarose gel showed extensive DNA fragmentation in testes of BPA-treated rats Significant increase in ROS level and decreased levels of CAT, GSH SOD and POD in the testis of BPA-treated group	not considered appropriate to measure DNA strand breaks in mature germ cells The comet protocol is shortly described, with no information on the number of analysed sperm cells per animal; sampling time not specified; cytotoxicity not evaluated; no positive control The DNA ladder assay is a biochemical method not validated for genotoxicity assessment	of information on test item purity	
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Table 9: *In vivo* dominant lethal assay
(OECD TG 478 was considered for the evaluation of the reliability)

Test system/Test object	Dose/Route	Information on the characteristics of the test substance	Results	Reliability/Comments	Relevance of the result	Reference
Dominant lethal test with male Holtzman rats (7 per group) Each treated male was mated with 2 females per week over a period of 8 weeks; the mated females were sacrificed on 15th day of gestation and uterine content examined <i>In vivo</i> comet assay in rat epididymal sperm (Table 8)*	Rats treated by oral gavage with BPA dissolved in ethyl alcohol and diluted in sesame oil, at dose levels of 10 µg/kg bw and 5 mg/kg bw once a day for 6 consecutive days Negative controls were treated with vehicle	BPA ~99% purity (Sigma Chemical Co.)	Positive Significant decrease in total implants/female and live implants/female, in females mated with males treated with 5.0 mg BPA/kg bw the fourth week and sixth week after treatment	Reliability: 2 No positive control No negative historical control Limited study design, with less analysable total implants and resorptions than recommended (OECD TG 478)	Limited	Tiwari and Vanage, 2013^{1*}

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Abbreviations

7-HF	7-hydroxyflavone
8-OHdG	8-hydroxy-2'-deoxyguanosine
ABT	1-Aminobenzotriazole
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AOPP	Advanced oxidation protein products
ATM	Ataxia-telangiectasia mutated
AU	Arbitrary units
BPA	Bisphenol A
BrdU	5-bromo-2-deoxyuridine
Bw	Body weight
CA	Chromosomal aberrations
CAT	Catalase
ChE	Cholinesterase
CHO cells	Chinese hamster ovary cells
CI	Cellular index
CYP450	Cytochrome P450 reductase
DCF	Dichlorofluorescein
DMSO	Dimethylsulphoxide
EM	Electron microscopy
ER	Oestrogen receptor
FACS	Fluorescence activated cell sorting
FISH	Fluorescence <i>in situ</i> hybridisation
Fpg	Formamide pyrimidine glycosylase
GGT	Gamma glutamyl transferase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
Hb	Haemoglobin
HDL	High-density lipoprotein cholesterol
HPLC	High performance liquid chromatography
HUVEC	Human umbilical vascular endothelial cells
i.p.	Intraperitoneal
KET	Ketoconazole
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein cholesterol
MDA	Malondialdehyde

MMC	Mitomycin
MMS	Methyl methane sulfonate
MPO	Myeloperoxidase
NAC	<i>N</i> -Acetyl-L-cysteine
NO	Nitric oxide
OTM	Olive tail moment
PBMC	Human peripheral blood mononuclear cells
PCE	Polychromatic erythrocytes
PCP	Pentachlorophenol
PHA	Phytohemagglutinin
POD	Peroxidase
RBC	Red blood cells
ROS	Reactive oxygen species
RT-PCR	Real time polymerase chain reaction
SCE	Sister chromatid exchange
SD	Sprague Dawley
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TC	Total cholesterol
TG	Test guideline
TP	Total protein
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labelling
WBC	White blood cells
WGS	Whole genome sequencing