on the basis of ligating a fluorescent chemical reporter probe to sites of DNA modification. Initially tested in oligonucleotide DNA, the method could be used to accurately detect enzyme-induced single-strand breaks with concentrations of oligonucleotide DNA as low as 200 pM. We similarly processed genomic DNA and could detect native levels of common DNA lesions such as oxidative modifications, apurinic sites and single-strand breaks. Furthermore, we quantified abasic sites and oxidative modifications in chemically exposed human cells. For the oxidizing agent potassium bromate, we detected a two-fold increase in oxidative damage for cells exposed to 50 mM potassium bromate compared to unexposed cells. For the anti-cancer drug irofulven, we could detect a five-fold increase in apurinic sites arising from alkylation-promoted depurination following exposure of a human cancer cell line to 15 μM drug. Fluorescence data were validated by parallel measurements with HPLC/MS. As a result of this study, we have established a convenient and effective general method for the rapid quantification of common DNA damage products in the human genome using a fluorescence-based ligation strategy. The method is anticipated to make DNA damage quantification more readily accessible for use in mechanistic toxicity studies, as a dosimeter for chemical exposure and risk assessment, and in precision medicine.

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P08-11
Fenhexamid promoted the initial step of metastasis in MCF-7 breast cancer cells via estrogen receptor and phosphoinositide 3-kinase pathway

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Fenhexamid (Fen) is used to eradicate the gray mold of fruits and vegetables. Fen is one of the most commonly used fungicides to prevent the gray mold of grapes. However, when the grapes are manufactured into wine, the Fen remains significantly in the wine rather than other fungicides. The current study has investigated the role of Fen and found the effect of Fen in human cancer. Here we studied further the malign influence of Fen in breast cancer models. We found that Fen was related to an increase in migratory ability and angiogenesis through regulation of estrogen receptor (ER) and phosphoinositide 3-kinase (PI3K) pathways in both ER-positive MCF-7 and ER-negative MDA-MB-231 breast cancer cells. MCF-7 and MDA-MB-231 cells were exposed to 17β-estradiol (E2, 10-9M), Fen (10-5M and 10-7M), ICI 182,780 (ICI; and ER antagonist, 10-8M) or/and Pictilisib (Pic; PI3K inhibitor, 10-7M). Then we conducted a migration assay, live-cell motility monitoring, trans-chamber assay, immuno-fluorescence, angiogenesis assay, tumor spheroid formation assay, and Western blot analysis. In MCF-7 cells, E2 and Fen increased cell migration through regulation of cell migration-related proteins but the expressions of N-cadherin and Vimentin, the marker genes of the EMT process which induces cell migration, remained unchanged. E2 and Fen, however, decreased the expression of N-cadherin and Occludin in the immunofluorescence assay and Western blot analysis. Also, Fen induced vessel formation in HUVEC cells and the formation of larger and denser tumor spheroids in MCF-7 cells. Then we confirmed the upregulated protein expression level of vascular endothelial growth factor (VEGF) and sex-determining region Y-box 2 (SOX2) after exposure to Fen through Western blot. Those results support the idea that Fen might be related to tumor progression. Therefore, we conclude that Fen plays an important role as an endocrine-disrupting chemical in breast cancer migration and metastasis through the regulation of ER and PI3K signaling pathways.

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P08-12
The surfactant co-formulant POEA in the glyphosate-based herbicide RangerPro but not glyphosate alone causes necrosis and ER stress in mammalian cell lines

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The toxicity of co-formulants present in glyphosate-based herbicide (GBHs) have long been demonstrated as a source of environmental and human health toxicity. In response to this, the European Union has banned the co-formulant polyoxyethylene tallow amine (POEA) from GBHs. Employing liquid chromatography-mass spectrometry, we identified the most commonly used POEA, known as POE-15 tallow amine (POE-15), in the widely used US GBH RangerPro. Cytotoxicity assays using human intestinal epithelial Caco-2 and hepatocyte HepG2 cell lines showed that RangerPro and POE-15 are far more cytotoxic than glyphosate alone. In addition, RangerPro and POE-15 but not glyphosate caused cell necrosis in both cell lines, and that glyphosate and RangerPro but not POE-15 caused oxidative stress in HepG2 cells. We further tested these pesticide ingredients in the ToxTracker assay, a system used to evaluate a compound's carcinogenic potential, to assess their capability for inducing DNA damage, oxidative stress and an unfolded protein response (ER stress). RangerPro and POE-15 but not glyphosate gave rise to ER stress. We conclude that the toxicity resulting from RangerPro exposure is thus multifactorial involving ER stress caused by POE-15 along with oxidative stress caused by glyphosate. Our observations reinforce the need to test both co-formulants and active ingredients of commercial pesticides to inform the enactment of more appropriate regulation and thus better public and environmental protection.

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P08-13
Quality evaluation and review of nanomaterial genotoxicity studies and data – a regulatory perspective

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Background: The number of publications in the field of nanogenotoxicology and the amount of genotoxicity data at several databases generated by EU funded projects have increased during the last

decade. The aim of this work was to investigate to what extent this information could be useful from a regulatory perspective.

Methods: Searches for publications on the genotoxicity of metal-containing nanoparticles (NP) and nanofibers (NF) were conducted in PubMed and Scopus and limited to OECD validated assays. We first evaluated the completeness of the physico-chemical (PC) characterization of the tested nanomaterials (NM), and the reliability of the studies according to the GUIDEnano quality assessment approach. Then, we created and applied a set of criteria that address assay-specific aspects for risk assessment purposes.

The NanoinformaTIX database, which contains data from nine EU initiatives plus the US caNanoLab, was searched for all entries regarding genotoxicity (validated or non-validated assays). The database was also evaluated for the data quality and the feasibility of data use from a regulatory perspective.

Results: From the original 620 literature search results, 194 *in vitro* and 92 *in vivo* studies fitted the scope of our review and were evaluated. More than half of studies were rejected due to an incomplete PC characterization of the test NM. Especially as concerns providing the purity of the NM and the size distribution during the exposure. Most of the studies with a complete characterization were also considered reliable. After applying the assay-specific criteria, 15% and 12% *in vitro* studies, and 18% and 33% *in vivo* studies on metal-containing NP and NF, respectively, passed all stages of the evaluation. The most common shortcomings included inadequate concurrent (cyto)toxicity measurement and treatment schedule, and lack of adherence to sample size or number of replicates. The most studied NM were TiO_2 NP, Ag NP, and carbon nanotubes (CNT). Mammalian gene mutation assays were clearly underused.

At the time of reviewing, a total of 41 genotoxicity-related data entries were identified in the NanoInformaTIX instance, being CNT, TiO_2, ZnO, and Ag NP the main identified NM. Half of the *in vitro* assays represented different versions of the comet assay, followed by the micronucleus (31%) and the mouse lymphoma (9%) assays. Likewise, the most popular *in vivo* method was the comet assay (94%). However, several names were found to indicate one given assay. NM were generally well-characterised, but characterisation data were not linked to toxicological results. Likewise, experiments were not linked regarding cytotoxicity and genotoxicity outcomes.

In view of these results, we provide recommendations on how to perform future genotoxicity studies that could be accepted and used by regulators.

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P08-14
Investigating the cytotoxic and genotoxic potential of carbon nanotubes in human cells *in vitro*

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Background: Carbon nanotubes (CNTs) are an important class of engineered nanomaterial (ENM) owing to their unique geometry, and physico-chemical features. Forecasted manufacture rates of CNTs will ultimately increase their inevitable human exposure. Despite focus *in vivo*, very little has been published which addresses the *in vitro* mutagenicity of these materials and moreover, their capacity to elicit genotoxicity in co-cultures. This research aimed to evaluate the cytotoxic, and genotoxic potential of two CNT types utilising: 1) the hypoxanthine-guanine phosphoribosyl transferase (*HPRT*) forward mutation assay, and 2) the *in vitro* cytokinesis-blocked micronucleus (CBMN) assay, following their respective Organisation for Economic Co-Operation and Development (OECD) test guidelines with minor modifications to adapt the assays for ENM testing.

Methods: The test materials NM400 and NM401 (Joint Research Centre (JRC), Italy) were dispersed, 1) according to the NanoGenoTox protocol; and 2) by pre-suspending and then dispersing in a Microfluidizer LM-10. For the *HPRT* assay, the materials were exposed to human lymphoblast (TK6) cells for 24 h (1–20 µg/ml) and 600 wells were scored for point mutations per concentration per biological replicate ($n = 3$). For the CBMN assay, monocultured human bronchial epithelial (16HBE14o-) cells and then a co-culture of 16HBE14o- with differentiated, human monocytic (dTHP-1) cells were exposed to CNTs (6.25–100 µg/ml) for 24 h. One thousand binucleated 16HBE14o- cells and 200 cells per replicate ($n = 2$) were scored for the frequency of micronuclei and replication index (RI), respectively. Proteomics experiments were performed using lung epithelial A549 cells and dTHP1 cells exposed to 1.5–50 µg/ml of CNTs for 24 h.

Results: The physico-chemical characteristics of the NM400 and NM401 appear affected by the suspension method. Raman spectra showed oxidative transformation on CNTs upon sonication, which is not apparent under high-shear preparation of suspensions. Despite this, no statistically significant ($p < 0.05$) cytotoxicity or genotoxicity was observed for either of the CNT types over the dose ranges applied in the *HPRT* and CBMN assays. Furthermore, varying the dispersion protocol had no impact on the hazard endpoint results. No major alterations to the proteomic profile of A549 nor dTHP-1 were observed following exposure to the test materials, regardless of sonication procedure utilised.

Summary: NM400 and NM401 undergo oxidative transformation upon sonication, however this did not induce significant toxicity, nor genotoxicity over a 24 h exposure period, regardless of the dispersion method utilised.

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P08-15
Assessing of *in vitro* genotoxicity testing strategy for T-2 mycotoxin

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The T-2 toxin (T-2) is a mycotoxin produced by fungi of the *Fusarium* genus, specifically it belongs to group A of trichothecenes. The T-2 is the most toxic of this group and it is frequently occurring in cereals, which is important to know its genotoxic potential. Therefore, the aim of this study is to explore the effect of T-2 on DNA damage on HepG2 cells. In order to achieve this goal, a battery of relevant *in vitro* toxicity test covering different genotoxic mechanisms is needed. Three *in vitro*