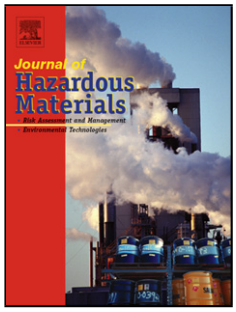

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Author: Alfredo Parrella Margherita Lavorgna Emma Criscuolo Chiara Russo Marina Isidori

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ECO-GENOTOXICITY OF SIX ANTICANCER DRUGS USING COMET ASSAY IN DAPHNIDS

Alfredo Parrella, Margherita Lavorgna, Emma Criscuolo, Chiara Russo, Marina Isidori*

Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Seconda Università di Napoli, Via Vivaldi 43, I-81100 Caserta, Italy

Highlights

- Eco-genotoxicity of six anticancer drugs was evaluated.
- *In vivo* Comet assay was performed on cells from whole crustaceans.
- Comet assay was the most sensitive genotoxicity test.
- *C. dubia*was used for the first time in the Comet assay.

* to whom correspondence should be addressed:

Marina Isidori

E-mail address: marina.isidori@unina2.it

Phone: +39 0823 274565

Abstract

The eco-genotoxicity of six anti-neoplastic drugs, 5-fluorouracil, capecitabine, cisplatin, doxorubicin, etoposide, and imatinib, belonging to five classes of Anatomical Therapeutic Classification (ATC), was studied applying the *in vivo* comet assay on cells from whole organisms of *Daphnia magna* and *Ceriodaphnia dubia*. F**or the first time, this test was performed in *C. dubia.* In addition, to have a wider genotoxic/mutagenic profile of the anticancer drugs selected, SOS Chromotest and Salmonella mutagenicity assay were performed. The Comet results showed that all drugs induced DNA damage, in both cladocerans, with environmental concern; indeed** Doxorubicin induced DNA damage in the order of tens of ng L^{-1} in both crustaceans, as well as 5-flurouracil in *C. dubia* and cisplatin in *D. magna*. In the SOS Chromotest all drugs, except imatinib, were able to activate the repair system in *Escherichia coli* PQ37 **while in the Salmonella mutagenicity assay,** doxorubicin was the only drug **able to cause direct and indirect frameshift and ~~missense mutations~~ base-pair substitution mutations.** Comet assay was the most sensitive tool of genotoxic exposure assessment, able to detect *in vivo* the adverse effects at concentration lower **than those** evaluated *in vitro* by bacterial assays .

Keywords

Anticancer drugs, genotoxicity, *Daphnia magna*, *Ceriodaphnia dubia*, mutagenicity.

Introduction

Anti-neoplastic drugs interfere directly or indirectly with the structure and functions of DNA and affect not only target cells, but also non-tumoural cells. Although the concentrations of these drugs in aquatic systems are very low, in the range of sub-ng L^{-1}-µg L^{-1} [1,2] the exposure of each living organism may be of environmental concern as its introduction is continuous and its presence relatively stable [3]. Data on environmental chronic toxicity of anticancer drugs is rather limited [4-6], the risk posed by these compounds is not easily predictable and it is difficult to establish the overall effects for aquatic organisms [7]. Jha [8] underlined that some ecotoxicological responses, as reproductive toxicity, are closely linked to the possibility to have some alterations in DNA such as strand breaks, DNA adducts, dimers and chromosomal aberrations. Therefore, **in addition to the evaluation of reproduction impairment at the whole organism level, the detection of genotoxic alterations could be of great ecotoxicological relevance especially when assessed on the same organism. The concurrent evaluation of toxicity and genotoxicity on the same bioindicator is even more important for compounds interacting with DNA with different modes of action and properties for which it is not possible** to establish a safe threshold [9]. **To date few studies have been performed to evaluate the eco-genotoxicity in aquatic organisms, *in vivo* or *in vitro* exposed to pharmaceuticals although high is the demand of bioassays focused on pharmaceuticals in general, and anti-neoplastic drugs in particular. Considering the ability of these compounds to interfere with the structure and function of DNA, it will be crucial to define their eco-genotoxicological effects to better identify the risk posed by these molecules.** For these reasons, the aim of the present study was to investigate the genotoxicity of six anti-neoplastic drugs in two freshwater crustaceans which represent key organisms of the aquatic food chain. The anti-neoplastic drugs studied belong to the five classes of the World Health Organization (WHO) Anatomical Therapeutic Classification (ATC) system according to the

respective chemical structure and therapeutic properties: 5-fluorouracil (5-FU) and its pro-drug capecitabine (CAP) are antimetabolites, cisplatin (CDDP) is a platinum-derived drug, etoposide (ET) is a topoisomerase II inhibitor, doxorubicin (DOX) a cytotoxic antibiotic and imatinib (IM) is a selective tyrosine kinase inhibitor **[7].** To assess the genotoxic potential of the six drugs selected, the comet assay was performed on cells from whole daphnids *in vivo* exposed to these compounds, *Daphnia magna* and *Ceriodaphnia dubia*, selected as biological model system. The comet assay is considered a sensitive tool for the detection of DNA strand breaking in natural biota [8] although few studies have been performed for the screening of the risk from environmental contamination in daphnids [10-12]. In addition to the Comet assay, the SOS Chromotest, a bacterial colorimetric assay based on the induction of a SOS repair system, was included to have a wider genotoxic profile of the anticancer drugs selected. Furthermore, since DNA damage and, especially strand breaks are potential pre-mutagenic lesions, **the direct and indirect** mutagenicity of the selected drugs was determined by the conventional **Salmonella mutagenicity assay** on two strains of *Salmonella typhimurium*, TA98 and TA100.

Materials and methods

Test compounds

5-FU (CAS: 51-21-8), CDDP (CAS: 15663-27-1), ET (CAS: 33419-42-0), and DOX (doxorubicin hydrochloride, CAS: 25316-40-9) were supplied by Sigma-Aldrich (Milano, Italy). CAP (CAS: 154361-50-9) and IM (CAS: 220127-57-1) were **provided** by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Dimethyl sulphoxide (DMSO) and compounds used for positive controls in bioassays were analytically pure (Sigma-Aldrich Chemicals, Italy).

Antineoplastic drug concentrations for mutagenicity/genotoxicity assays

The stock solution of each chemical was prepared in deionized water (Elix 10, Millipore, Milan, Italy), except for ET that was previously dissolved in DMSO and further diluted in deionized

water. The test solutions were prepared by serial dilutions of the stock solutions immediately before tests. In the Comet assay, the concentrations started from at least 1/10 the respective acute EC50 value previously estimated from the dose response curves of the single compounds reported in a previous study [6] and arranged in a geometric series with a factor 10. DMSO percentage was lower than 0.01% v/v in ET test solutions. Both in the Salmonella mutagenicity assay and in the SOS Chromotest, anticancer drugs were tested starting from concentrations of environmental concern up to concentrations in the same order of magnitude found in the literature [4].

Test organisms for Comet assay

The crustaceans *Daphnia magna* Straus and *Ceriodaphnia dubia* were **obtained** from laboratory cultures (starting organisms from Aquatic Research Organisms, Inc., Hampton, NH, USA). *D. magna* was maintained in moderately fresh water (hardness 170 mg L^{-1}, expressed as $CaCO_3$) [13] and *C. dubia* in ISO medium (hardness 250 mg L^{-1} expressed as $CaCO_3$) [14]. The stock cultures were incubated at 20 ± 1 °C for *D. magna* and 25 ± 1 °C for *C. dubia*, with a 16:8 h light:dark cycle (500 lux). A combination of 5 g L^{-1} each of yeast (*Saccharomyces cerevisiae*), alfalfa, and flake food, in addition to the unicellular green alga *Pseudokirchneriella subcapitata* (10^8 cells mL^{-1}), provided suitable nutrition for crustaceans. The organisms to expose to anticancer drugs were less than 24h old and coming from the second to the fifth brood, obtained by parthenogenesis.

Comet assay

The Comet assay was performed on cells coming from whole organisms. About 20 neonates were placed in glass beakers and exposed to drug solutions. After 24h, the organisms treated were placed in 1 mL of phosphate-buffered saline (PBS) containing 20 mM ethylene diamine tetra-acetic acid (EDTA) and 10% dimethyl sulfoxide (DMSO) and then disintegrated by serial pipetting according to [10]. Treatment with hydrogen peroxide was used as the positive control: H_2O_2

concentrations for *D. magna* were 1 and 10 µM, for *C. dubia* 0.1 and 1 µM. After centrifugation (5 000 rpm), the cells were spread onto microscope slides pre-coated with normal agarose and subjected to the alkaline comet assay [15]. Viability was checked by trypan blue staining. The cells were lysed for 1h in 10 mM Tris, 100 mM EDTA, 2.5 M NaCl, 10% DMSO, 1% Triton X-100 (pH 10). DNA unwinding was carried out for 20 min at 4°C in alkaline conditions (300 mM NaOH, 1 mM EDTA, pH≥ 13). The electrophoresis were performed at 4°C under an electrical current of 400 mA (25V) for 20 min. The slides were neutralized (TrisHCl 0.4 M), dehydrated in 70 % ethanol, stained with 50 µL ethidium bromide (10 µg L^{-1}) and then analysed using a fluorescence microscope (400X magnification, Eclipse 50*i*, Nikon, Kanagawa, Tokyo). The cytostatics were tested in three independent assays, about 50 cells per slide (2 slides per each of four/six concentrations) were scored using Comet assay IV image analysis software (Perceptive Instrument, UK). The test parameter considered was the % DNA in tail (tail intensity). Comet assay results were analysed for significance from negative control using ANOVA and Dunnett's multiple comparison tests. Results were also examined in a multiple comparison procedure with Tukey's HSD (Honestly Significantly Different) test to verify differences between compound concentrations ($p < 0.05$).

SOS Chromotest

The SOS Chromotest [16] was performed on *Escherichia coli* PQ37 strain. This strain carried a *sfiA*::*lacZ* fusion gene and a deletion of the normal *lac* region; thus, the *β*-galactosidase activity was strictly dependent on *sfiA* expression, which increased in response to specific DNA damaging agents. Culture of *E. coli* was incubated with five cytostatic concentrations, positive control (4-nitroquinoline-N-oxide) and negative control at 37°C. After 2h of incubation, *β*-galactosidase and alkaline phosphatase (constitutive in *E. coli* PQ37) activities were measured as ortho-nitrophenol and 4-nitrophenyl concentration at 420 nm (SpectraFluor, Tecan, Switzerland). The results were expressed as induction factor (IF) for each dilution of compound, defined as $IF = R/R_0$, in which

R=*β/ρ* where *β* represents *β*-galactosidase activity and ρ, phosphatase alkaline activity, R_0 is the ratio measured in the negative control. A compound was considered as an SOS repair system inducer in *E. coli* if the IF was higher than 2, the β-galactosidase activity was significantly increased compared to the negative control and the induction factor versus concentration showed a clear dose-effect relationship.

Salmonella mutagenicity assay

The mutagenicity of the **cytostatic drugs** was evaluated on two *Salmonella typhimurium* strains, TA98 strain for frame-shift mutations, and TA100 strain for base-pair substitutions. Four or five cytostatic concentrations (0.1mL) **and 0.1 mL of medium containing 10^8 cells were incorporated into agar and plated with 0.5 mL of S9 mix (metabolic activation) to detect indirect mutagenic effects or 0.5 mL of phosphate buffer to detect direct mutagenic effects [17].** Sodium azide for TA100 and 2-nitrofluoren for TA98 were used as positive controls of direct mutagenicity, while cyclophosphamide for TA100 and 3-methyl-colantrene for TA98 were used as positive control of indirect mutagenicity. **Three plates for each concentration were incubated at 37°C in the dark.** After 72h the number of His^+ revertants was counted, the mean number of revertants induced by the sample **was** compared to the mean of spontaneous revertants in the negative control using ANOVA and Dunnett's multiple comparison tests. **Three independent experiments were performed.**

Lab work was performed in compliance with current safety guidelines and the use of personal protective equipment. All people involved in the experiments with anticancer drugs were aware of and followed the waste disposal rules and procedures for proper disposal of hazardous wastes.

Results and discussion

Comet assay plays an important role in eco-genotoxicological detection, and although the species reproduction is the main parameter considered in ecotoxicology, it is central to understand which biological factors influence the normal reproductive pattern of wild species. Since Comet assay allows **a rapid and sensitive** detection of a broad spectrum of DNA damage **[15]**, **in this study it was performed on cells from whole organisms,** representative of the aquatic chain such as *D. magna* and *C. dubia,* **usually used in toxicity testing.** At the applied compound concentration ranges, the cells of the exposed organisms were viable as confirmed by Trypan blue staining. As genotoxicity sample testing, positive controls were performed (Figure 1). ***D. magna* and *C. dubia* were both sensitive to the DNA damage induced by the six anticancer drugs selected and the results** are shown in Figures 2 and 3, respectively.

Under the same exposure conditions, *C. dubia* was slightly more sensitive than *D. magna* to the damage induced by the anti-neoplastic drugs tested, except for CDDP **and CAP** which reported a % DNA in tail (tail intensity) significantly different from the negative control starting from 0.3 **and 1.2 x10^2 µg L^{-1}, respectively, one order of magnitude higher than *D. magna* (0.01 µg L^{-1} for CDDP and 22.5 µg L^{-1} for CAP).** CDDP and DOX were the most active genotoxic compounds on *D. magna* followed by ET, 5-FU, IM and CAP. On *C. dubia*, 5-FU and DOX induced a statistically different increase of DNA strand breaks starting from 0.06 and 0.05 µg L^{-1}, respectively while ET, CDDP and IM showed DNA damage at a concentration of one order of magnitude higher than 5-FU and DOX (Figure 3). CAP was genotoxic at concentrations clearly higher than **those of** the other anticancers. Significant differences among concentrations were found for $p<0.05$ at Tukey's HSD multiple comparison test, indicating a good dose-response relationship for both organisms. **5-FU DNA damage** has been recently documented **also** using Comet assay in haemocytes of freshwater mussels *Unio pictorum* and *Unio tumidus in vivo* exposed to this antimetabolite [18] although *D. magna* and *C. dubia* were more sensitive in the present study. **Genotoxicity of 5-FU was also**

supported by the study of Grisolia and Cordeiro [19] performed in different species of fish using micronucleous assay. Just recently, IM eco-genotoxicity was assessed in higher plants. Pichler et al. [20] demonstrated the induction of DNA damage in *Tradescantia paludosa*, expressed as increase of micronuclei (MN) at concentrations ≥ 5.9 mg L^{-1} while in the root tip cells of *Allium cepa* a significant induction of MN was detected at concentrations starting from 0.6 mg L^{-1}. The same authors, comparing the genotoxic effect of ET, CDDP and 5-FU in *T. paludosa* and *A. cepa*, found that IM was 10-100 times less effective than CDDP and ET but more potent than 5-FU. These results partially agree with those we found testing *D. magna.*

The genotoxic effect of anticancer drugs shown by the Comet assay is obviously linked to the mode of action of such drugs. DOX and CDDP can cause DNA double-strand breaks, **the former inhibiting the function of topoisomerase II, the latter inducing interstrand and intrastrand cross-links by DNA adducts**. Although the exact mechanism of double strand breaks formation is unclear, cross-links probably distort the shape of the DNA double helix causing DNA damage during gene expression and replication [21,22]. Matuo et al. [23] showed that 5-FU induced DNA double-strand breaks measuring the appearance of the γH2AX phosphoepitope that is a phosphorylated form of the histone H2AX, a specific marker for the detection of these breaks. ET, an inhibitor of topoisomerase II, is a potent inducer of breakage of DNA strands **as shown by** Smart et al. [24]. Unexpectedly, although IM does not act directly on DNA, in the present study it was found genotoxic probably because, as reported by **Fabarius et al. [25], it induces centrosome aberrations *in vitro* and then genetic instability**. The slightly genotoxic potential of CAP could be due to single strand breaks caused by an incomplete synthesis of DNA [26].

Table 1 and Table 2 show the results obtained from the evaluation of tail intensity in the Comet assay, expressed as NOAEC (No Observable Adverse Effect Concentration) and LOAEC (Lowest Observable Adverse Effect Concentration) in comparison to the respective chronic toxicity data

reported by Parrella et al. [6], expressed as NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration). From the findings here shown, the tail intensity seems to be a sensitive measurement for exposure effects in the two crustaceans since both chronic LOEC and NOEC were found at higher concentrations. **Among the pharmaceuticals investigated the highest difference (three orders of magnitude) comparing LOAEC and LOEC values was found for ET in both organisms.** In view of the fact that anticancer drugs are detected in aquatic systems at very low concentrations, sometimes below the lower limit of detection, it is essential to have a sensitive bioassay able to detect adverse effects in key organisms of the aquatic chain to integrate the ecotoxicological information of genotoxic compounds. **Comet assay showed high sensitivity even though it employed a mixture of cell types from whole animals without the ability to detect an organ-specific response.**

To have a wider genotoxic profile of the anticancer drugs selected, ~~and to~~ ~~compare Comet assay results to other eco-genotoxic responses, the anti-neoplastic drugs~~ **these latter** were also subjected to the SOS Chromotest. All anticancer drugs, except IM, were able to activate the SOS repair system in *E. coli* PQ37 although the effective concentrations were generally in the order of mg L^{-1} (Table 3). DOX was found genotoxic at the concentration of 0.63 mg L^{-1} and this finding agrees with that of Zounkova et al. [4] who showed effective concentrations quite similar to those found in the present study also for 5-FU and ET. **CDDP was recognized as** a potent SOS inducer at concentrations in the order of hundreds of mg L^{-1}[27], in the present study was active from 1.25 mg L^{-1}, one order of magnitude higher than the concentration reported by Zounkova and co-authors [4]. CAP was genotoxic starting from 75 mg L^{-1}, confirming also in the SOS Chromotest a lower potency. **The results obtained in this test agree with the Comet assay results except for IM since DNA strand break damages, detectable by Comet assay, might also induce the SOS response, as shown by Yasunaga and co-authors [28] in the Salmonella umu test. Nevertheless,**

the concentrations able to induce the SOS repair system were higher (from two to five order of magnitude) than those causing genotoxicity in the Comet assay. Probably, IM did not induce the SOS response because it does not cause a direct damage on DNA even if a genetic instability was detected by the Comet assay.

The mutagenic activity of the anticancer drugs selected, obtained by the standard plate incorporation assay, is shown in Table 4. **This test** is the most frequently used mutagenesis assay even for surface water monitoring [29]. The drawback of this test in detecting mutagenesis of anti-cancer drugs is that it requires high concentrations to obtain positive results [9]. Our results confirmed what said above showing that 5-FU, CDDP, DOX and IM exhibited a mutagenic activity without metabolic activation at relatively high concentrations. In TA98, IM and DOX induced frameshift mutations with a clear concentration-related increase in the number of revertant colonies, starting from 2.20 and 2.50 mg L^{-1}, respectively. Evidence of a less mutagenic **activity** in this strain was shown by 5-FU and CDDP. In TA100 strain IM and CDDP showed **mutagenicity** at concentrations lower than those found in TA 98 while DOX and 5-FU confirmed their **activity** at the same concentrations. ET did not exhibit any evidence of mutagenic **potential** in both salmonella strains while CAP in TA100 showed just a weak response at the highest concentration tested. In the presence of metabolic activation, DOX was positive in both strains while CAP and IM only for TA98 and TA100, respectively.

Comparing the results of mutagenesis to the literature, the absence of mutagenicity of ET confirmed the result of previous studies [9] while the high mutagenic **activity** of IM was in contrast to earlier *Salmonella*/microsome assays [30]. In support of the mutagenicity found for CDDP, 5-FU and DOX there are previous studies where these anticancer **drugs** resulted to induce *His*$^{+}$ revertants in *S. typhimurium* TA98 and TA100 strains [31,32]. The mutagenic effect of cytostatics depends on their mode of action. Surprisingly, IM was mutagenic on both strains in absence of

metabolic activation although it does not interact with DNA, as above mentioned, nevertheless, after hepatic metabolism, less active N-demethylated metabolites are produced [33]. **The positivity** of DOX on both strains may be explained by its intercalation of DNA, **probably** linked to oxidative DNA damage through the formation of free radicals [31]. **5-FU was exclusively found direct mutagen as** it could be subjected to a metabolic inactivation according to Zounkova et al. [4]. On the contrary, CAP, prodrug of 5-FU, induced **frame-shift** mutation in TA98 in presence of S9 mix probably because CAP is metabolized to 5'-deoxy-5-fluorocytidine, 5-FU and other active metabolites, as demonstrated by *in vitro* kinetic analyses [34]. To summarize, the results here reported indicate that Comet assay on *in vivo* organisms exposed to toxicants is able to detect genotoxic alterations also in the case of drugs not directly acting on DNA giving more information than traditional tests do.

Conclusions

At the best of our knowledge, this is the first study in which Comet assay was used not only to detect genotoxicity of some anticancer drugs in daphnids, but also it was the first time that this test was performed in *C. dubia*. Considering the widespread abundance of *C. dubia*, **its use in *in vivo* genotoxicity testing** could be a further support as representative invertebrate species in the environmental risk assessment. According to our results, the evaluation of DNA damage after 24 h exposure of whole organisms could be considered an early biomarker of the effect on survival and/or reproductive toxicity representing a useful tool for environmental monitoring and risk assessment of anticancer drugs. Indeed, all selected drugs induced an increase of DNA damage in cells of *D. magna* and *C. dubia*, **at concentrations of environmental concern and lower** than those able to induce *His*$^{+}$ revertants in **the Salmonella mutagenicity assay** and to activate SOS repair system in *E. coli* PQ37. Furthermore, in the sewer network and in environment, the concurrent

presence of different molecules, metabolites and transformation products require an accurate evaluation of the adverse effects of genotoxic pollutants to better assess their real impact on aquatic biota and to know whether environmental exposure to such compounds pose a risk to wildlife and human health. **For this purpose, Comet assay on key organisms of the aquatic chain could be an advantage to detect early adverse effects of compounds at very low concentrations and in a short time as well as to assess the overall genotoxic loads in the aquatic environment.**

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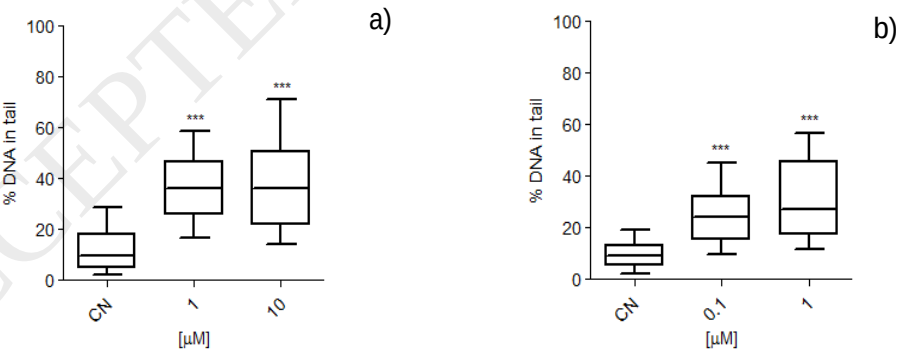

Figure 1

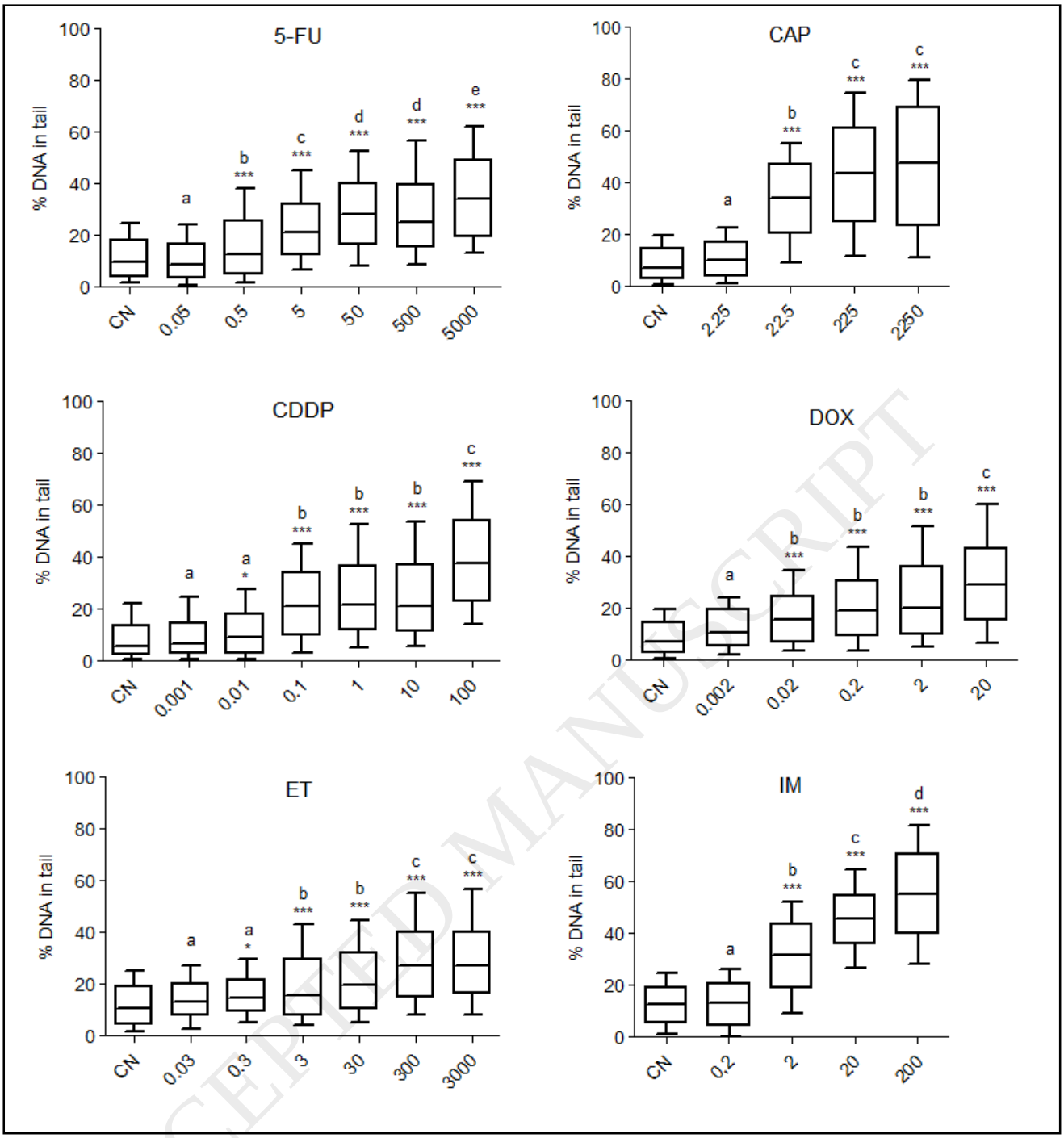

Figure 2

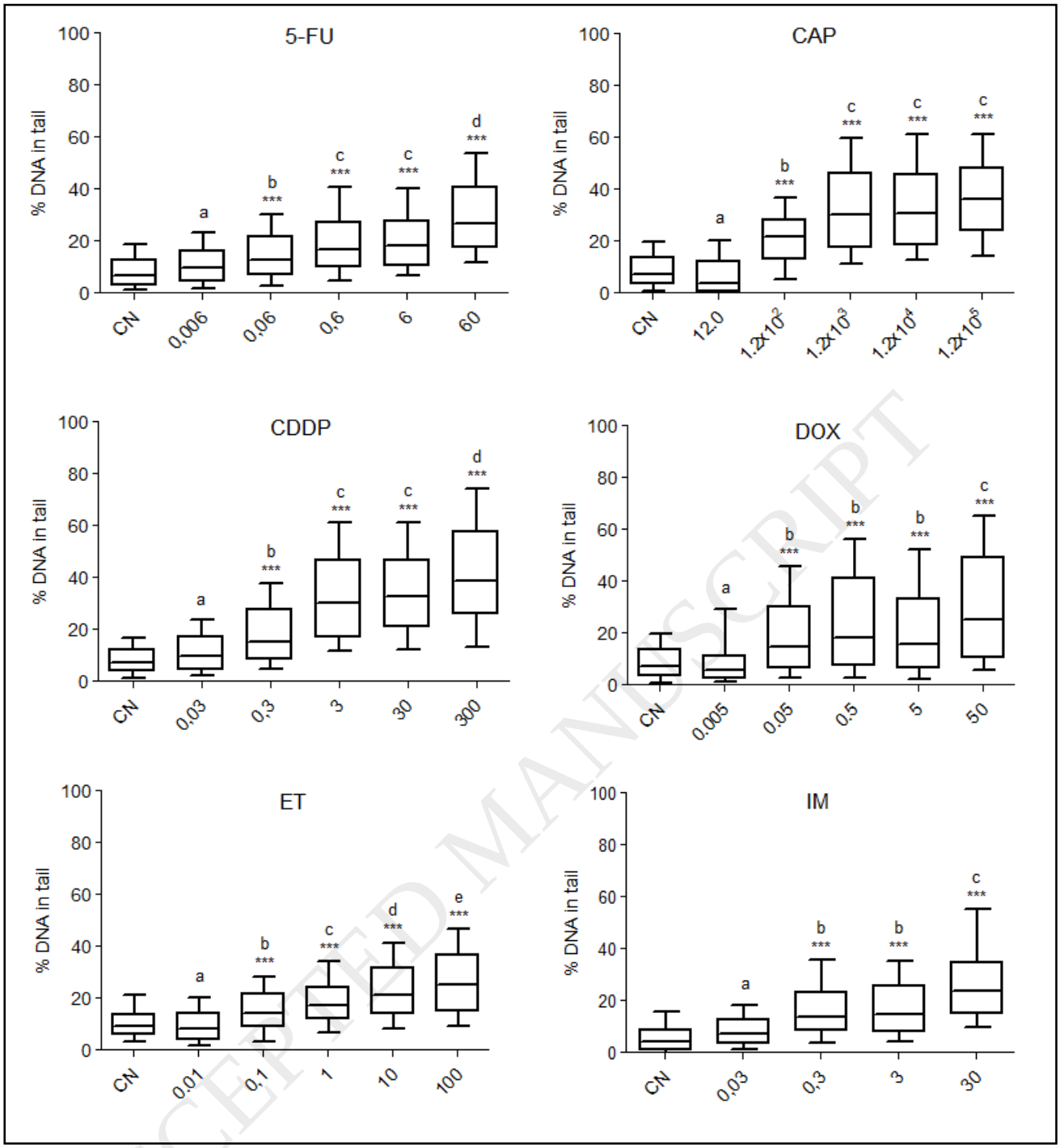

Figure 3

Figure captions

Figure 1: Effects of H_2O_2 (positive control) on induction of DNA strand breaks in *D. magna* (a) and *C. dubia* cells (b). Results, expressed as % DNA in tail, are from three independent experiments (300 nuclei). Data are presented as quantile box plots. Significant difference from control was determined with Dunnett's test ***p<0.0001.

Figure 2: Effects of 5-FU, CDDP, ET, IM, DOX and CAP on induction of DNA strand breaks in *D. magna* cells. Results, expressed as % DNA in tail, are from three independent experiments (300 nuclei). Data are presented as quantile box plots. **Each x-axis shows the concentrations of the drug, expressed in µg l^{-1}.** Significant difference from control was determined with Dunnett's test *p<0.05, ***p<0.0001. Different letters mean significant differences for $p < 0.05$ among concentrations expressed in µg l^{-1} (Tukey's HSD multiple comparison test).

Figure 3: Effects of 5-FU, CDDP, ET, IM, DOX and CAP on induction of DNA strand breaks in *C. dubia* cells. Results, expressed as % DNA in tail, are from three independent experiments (300 nuclei). Data are presented as quantile box plots. **Each x-axis shows the concentrations of the drug, expressed in µg l^{-1}.** Significant difference from control was determined with Dunnett's test ***p<0.0001. Different letters mean significant differences for $p < 0.05$ among concentrations expressed in µg l^{-1} (Tukey's HSD multiple comparison test).

Table 1: Estimation of **No Observable Adverse Effect Concentration** (NOAEC) and **Lowest Observable Adverse Effect Concentration** (LOAEC) values (µg l^{-1}) obtained from the evaluation of tail intensity in *D. magna* (24h exposure) and **No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC)** values (µg l^{-1}) obtained from chronic toxicity testing in the same organism (21 days of exposure).

Compound	NOAEC Comet assay	LOAEC Comet assay	NOEC* Chronic test	LOEC* Chronic test
5-FU	0.05	0.5	2.06	6.17
CAP	2.25	22.5	1900	6100
CDDP	0.001	0.01	1	3
DOX	0.002	0.02	-	-
ET	0.03	0.3	111.1	333.3
IM	0.2	2	2.98	9.54

- Not Available

*Parrella et al., 2014

Table 2: Estimation of **No Observable Adverse Effect Concentration** (NOAEC) and **Lowest Observable Adverse Effect Concentration** (LOAEC) values (µg l^{-1}) obtained from the evaluation of tail intensity in *C. dubia* (24h exposure) and **No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC)** values (µg l^{-1}) obtained from chronic toxicity testing in the same organism (7 days of exposure).

Compound	NOAEC Comet assay	LOAEC Comet assay	NOEC* Chronic test	LOEC* Chronic test
5-FU	0.006	0.06	2.22	6.67
CAP	12	$1.2x10^2$	600	1900
CDDP	0.03	0.3	4.57	14.65
DOX	0.005	0.05	-	-
ET	0.01	0.1	97.6	312.5
IM	0.03	0.3	0.27	0.87

- Not Available

*Parrella et al., 2014

Table 3: Genotoxicity in the SOS Chromoteston *E.coli* PQ37, expressed as induction factor (IF). Data is means ± standard deviation of three independent experiments. A compound is considered genotoxic(in bold) when IF is higher than 2 and the induction factor versus concentration shows a clear dose-effect relationship.

SOS Chromotest		
Compound	Concentration (mgl^{-1})	IF
4-NQNO	1.0	**2.09±0.02**
	5.0	**12.20±2.08**
5-FU	0.63	1.28±0.04
	1.25	1.50±0.17
	2.50	**2.10±0.24**
	5.0	**2.52±0.37**
	10	**3.54±0.18**
CAP	18.75	1.75±0.30
	37.5	1.81±0.35
	75	**2.05±0.25**
	150	**2.28±0.26**
	300	**2.65±0.01**
CDDP	0.63	1.26±0.03
	1.25	**1.92±0.25**
	2.50	**2.37±0.25**
	5.0	**2.66±0.15**
	10	**3.03±0.03**
DOX	0.31	1.72±0.04

	0.63	**2.13±0.04**
	1.25	**2.41±0.17**
	2.50	**2.60±0.24**
	5.0	**2.52±0.37**
	0.63	1.88±0.04
	1.25	**2.16±0.17**
ET	2.50	**2.38±0.24**
	5.0	**2.53±0.37**
	10	**2.67±0.18**
	0.20	0.88±0.23
	0.70	0.96±0.34
IM	2.20	0.76±0.35
	6.60	1.01±0.30
	20.00	1.34±0.33