# Genotoxicity of nitroaromatic compounds in zebrafish embryos

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## Introduction

**Degradation processes at munition dumpsites expose marine organisms such as fish to various nitroaromatic contaminants. Trinitrotoluene (TNT) and its metabolites are known for its mutagenic effects[1] but genotoxicity of these compounds remains still unclear[2,3]. Here we test genotoxicity of nitroaromatic compounds to fish by using the comet assay as experimental setup. This study clearly proves that TNT and its degradation products pose a genotoxic risk to fish.**

## Methods

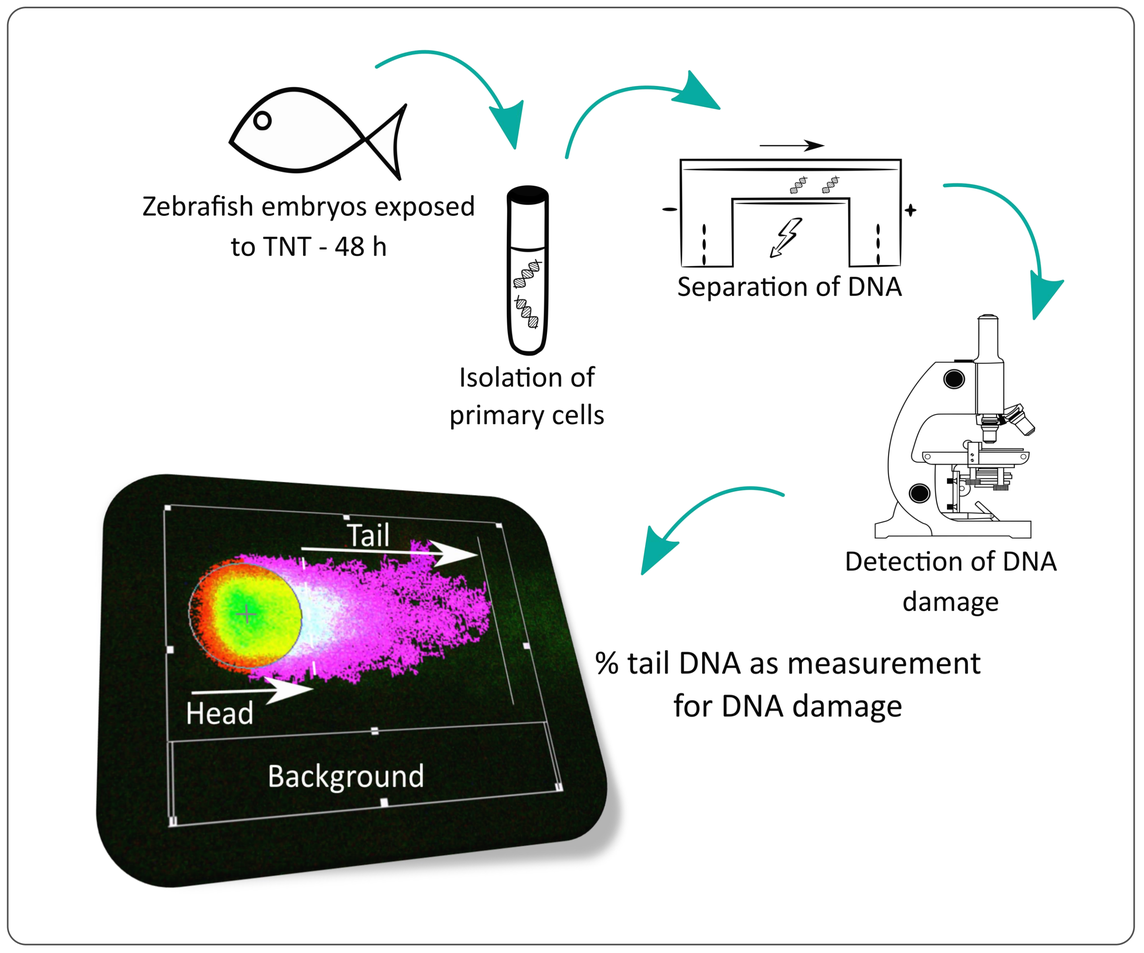


**Figure 1:** Quantification of genotoxicity in Zebrafish embryos by using the alkaline comet assay[4]. Evaluation of DNA damage by calculating the percentage of DNA in the tail. Head- and tail-intensities, as well as background intensities were determined using the CASP Software[5].

## Results & Discussion

- After exposure for 48 h even the lowest tested concentration of 2-ADNT (1 mg/l) and TNT (0.1 mg/l) led to a significant increase of genotoxicity in zebrafish embryos compared to the control (see Figure 2). Comparable effects were observed in experiments with 4-ADNT.
- No clear dose-response relationship could be found.
- The higher solubility of TNT in water compared to its primary metabolites could explain the higher genotoxic potential of TNT.
- Since TNT is rapidly metabolized *in vivo*[6], the genotoxicity of 2-ADNT and 4-ADNT plays a crucial role in explaining adverse effects on fish in the marine environment.

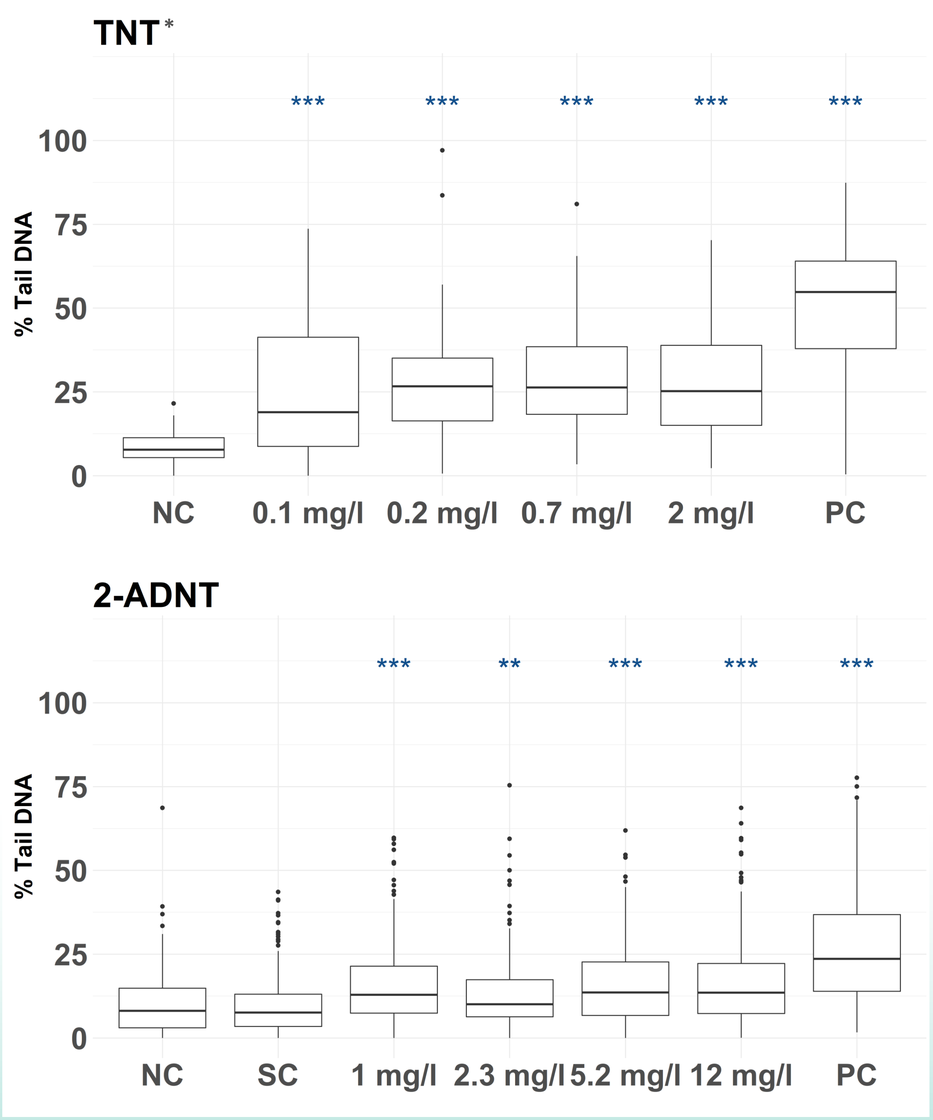


**Figure 2:** Genotoxicity of TNT and 2-ADNT in primary cells of zebrafish embryos after *in vivo* exposure for 48 h. DNA damage is expressed as percentage of DNA in the tail (% tail DNA). Dilution water was used as negative control (NC), 0.1 % DMSO as solvent control (SC) and hydrogen peroxide (48 hpf embryos were exposed for 15 min) as positive control (PC). 80 comets from two slides per treatment were evaluated, 3 replicates each. Asterisks express significance, determined by GLM using gamma distribution.
* TNT genotoxicity data consists only of 1 replicate.

## Conclusion

- **TNT as well as their primary degradation products (ADNT's) are clearly genotoxic for fish.**
- **Genotoxicity of TNT is more than 6 times higher than that of degradation products (see Figure 3).**

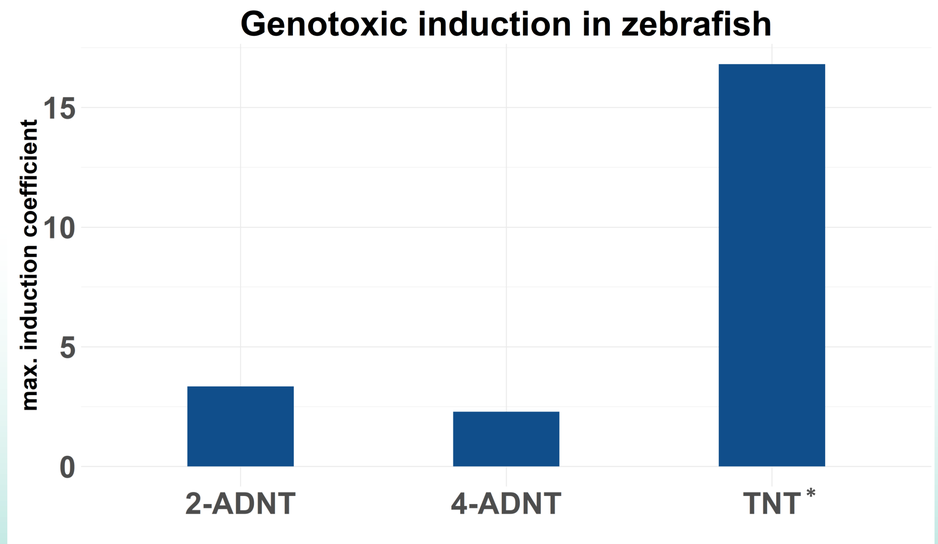


**Figure 3:** Maximum genotoxic induction coefficient of TNT, 2-ADNT and 4-ADNT in primary cells of zebrafish embryos after *in vivo* exposure over 48 h. The genotoxic induction was calculated by dividing the maximum median tail moment of the treatment by the median tail moment of the corresponding control.
* TNT genotoxicity data consists only of 1 replicate.

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