

Preliminary final report

Supplements

NORMAN WG-2 Bioassays
**JPA COLLABORATIVE TRIAL ON BIOASSAYS FOR NEUROTOXICITY
TESTING**

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on behalf of all the participants of the JPA

Since a face-to-face meeting in Sept 2020 on the discussion of the results obtained by the interlab study in a broader context and the publication on the results had to be postponed due to the COVI-19 situation to Summer 2021 this report is only a preliminary final report on the JPA

S1. Info Sheet – Preparation of the environmental matrix for NeuroBox ring test

Sampling:

The sample was taken in January 2018, from Spittelwasser, Germany. Approximately 100 L of water was Extracted on-site by large volume solid phase Extraction (LVSPE) on chromabond HRX, a hydrophobic polystyrene-divinylbenzene copolymer (Macherey-Nagel, Düren, Germany). The loaded HRX was freeze-dried and Extracted with ethyl acetate and methanol. The Extract was concentrated and recovered in to 100 mL of final volume in methanol (concentration factor of 1000).

Preparation:

- In each vial, 4 mL of Extract (REF 1000) was evaporated near to dryness
- Samples were then dissolved into 100 µL of DMSO (Final REF: 40000)
- In order to achieve a final REF of 20000 it is necessary to add other 100 µL of DMSO*

*The final REF of 20000 was chose since the max amount of solvent in the FET assay is 0.1%. This means that for a 20 mL exposure system, it is necessary to use a max amount of 20 µL of Extract. This is a dilution of 1000 and a final REF of 20 in the exposure media.

S2. Results of participants

EAWAG (Light-dark-transition-test)

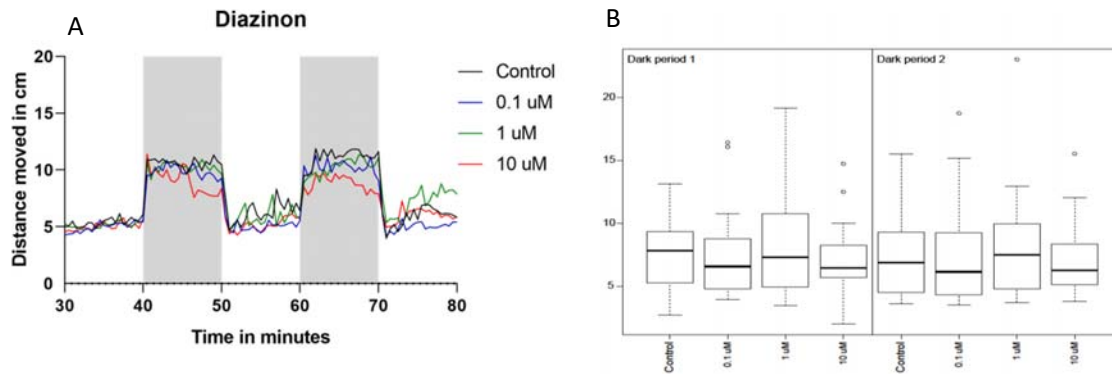


Figure S1: (A) Total distance moved (cm) by embryos exposed to three different concentrations of Diazinon in a light/dark transition test after 120 hours exposure. (B) The boxplots additionally show the means of distance travelled (cm) for each treatment and both dark periods separately.

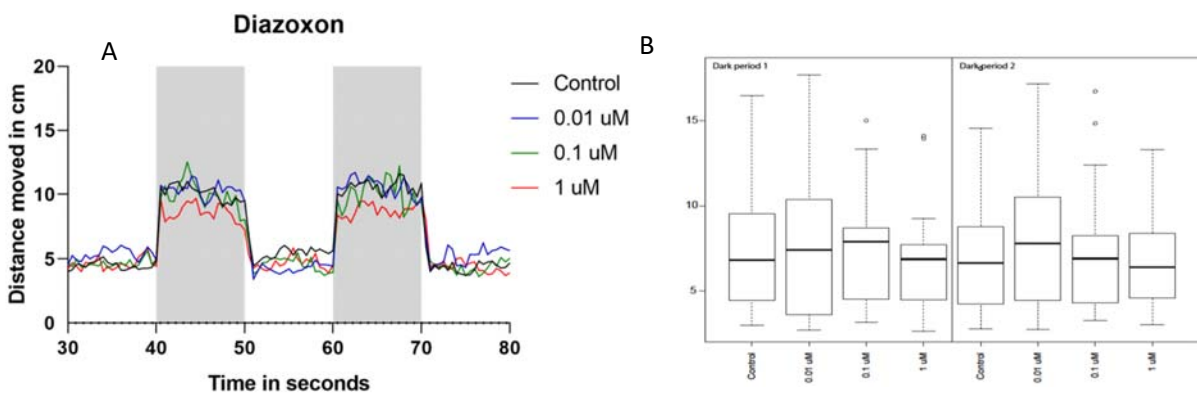


Figure S2: (A) Total distance moved (cm) by embryos exposed to three different concentrations of Diazoxon in a light/dark transition test after 120 hours exposure. (B) The boxplots additionally show the means of distance travelled (cm) for each treatment and both dark periods separately.

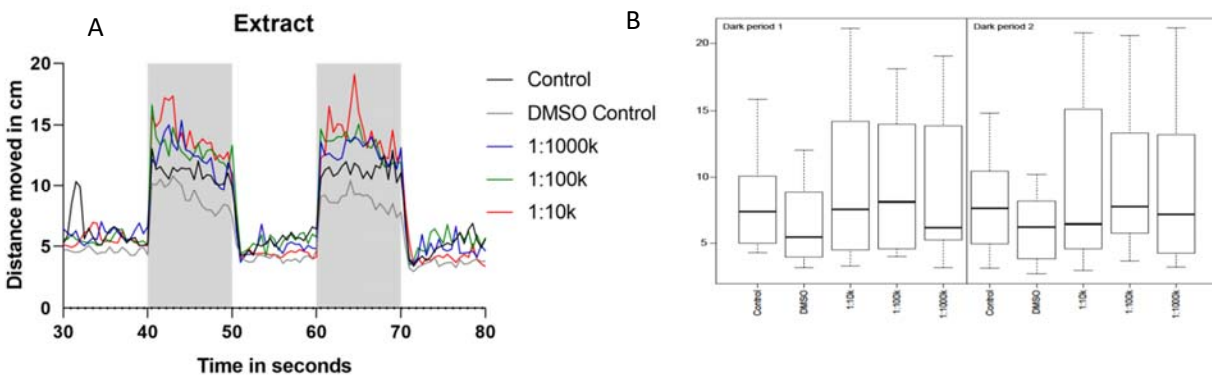


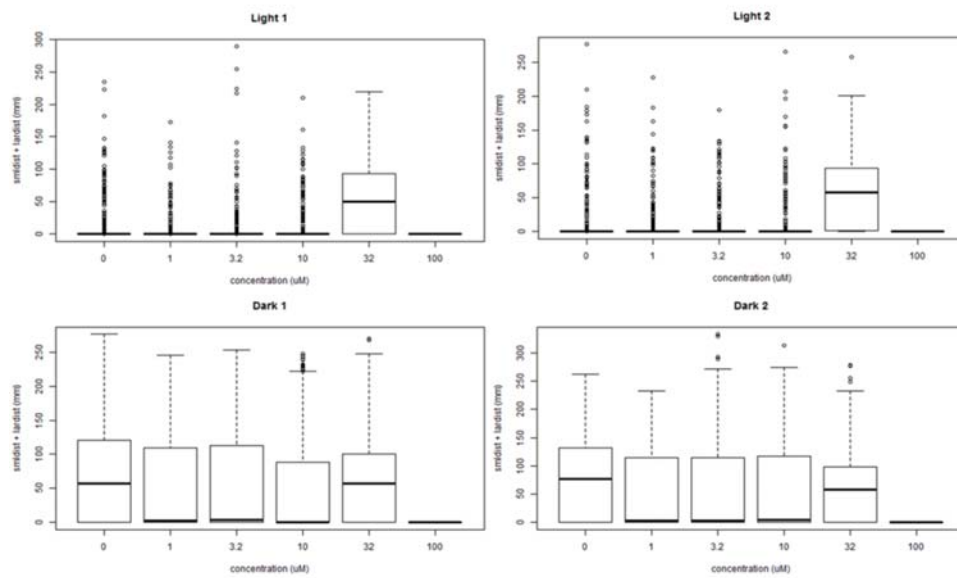
Figure S3: (A) Total distance moved (cm) by embryos exposed to three different concentrations of an Extract in a light/dark transition test after 120 hours exposure. (B) The boxplots additionally show the means of distance travelled (cm) for each treatment and both dark periods separately.

UFZ

Light-dark-transition-test (LMR)

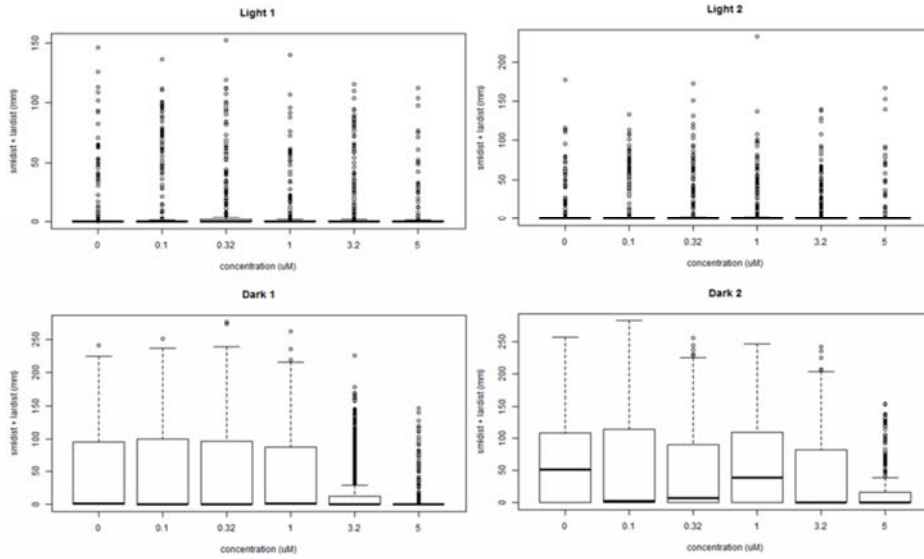
a)

LMR Results - Diazinon



b)

LMR Results – Diazinon oxon



c)

LMR Results – Environmental sample

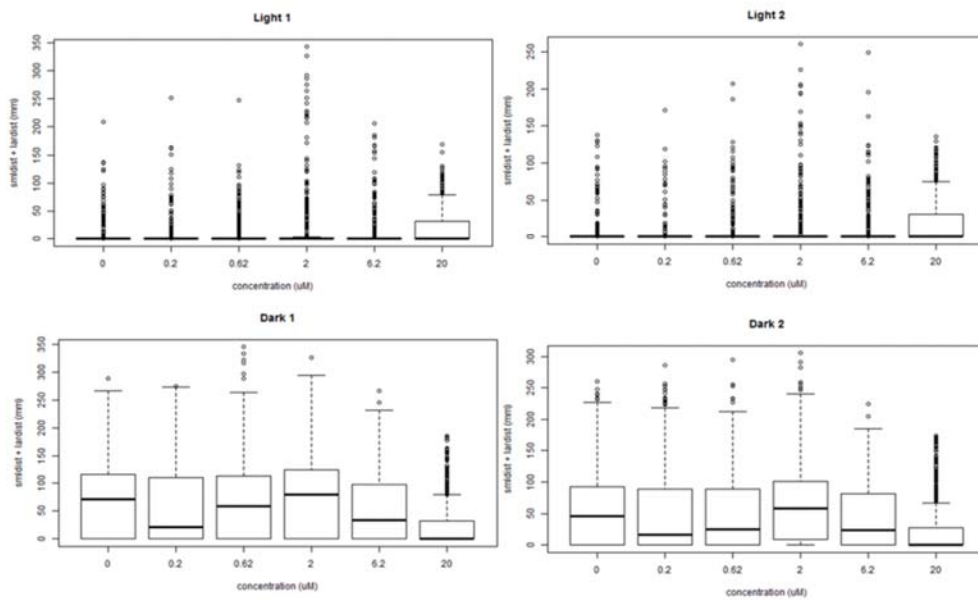


Figure S4: Total swimming distance (mm) of 16 embryos in a dark/light transition test after 96 exposure to a) Diazinon, b) Diazoxon and c) Extract. Total distance represents the sum of small distance

(movements ≤ 0.2 mm) and large distance (movements ≤ 2 mm) using an internal KNIME workflow. Boxplots represent the median movement x minute measurement.

Spontaneous movement

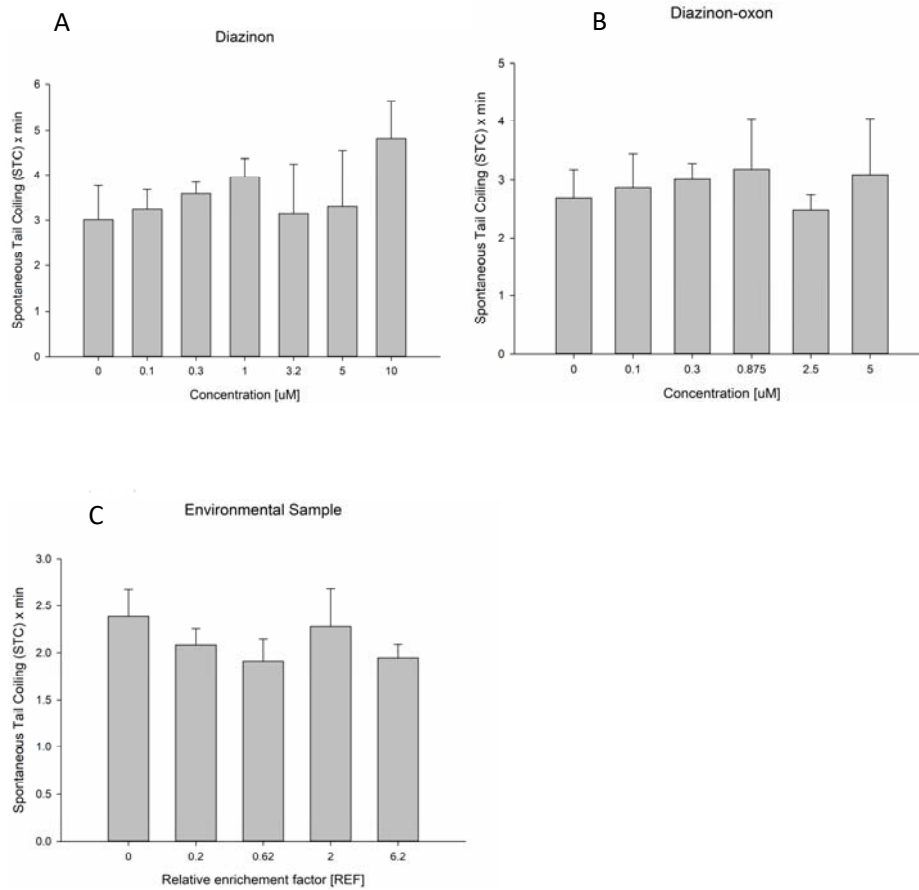
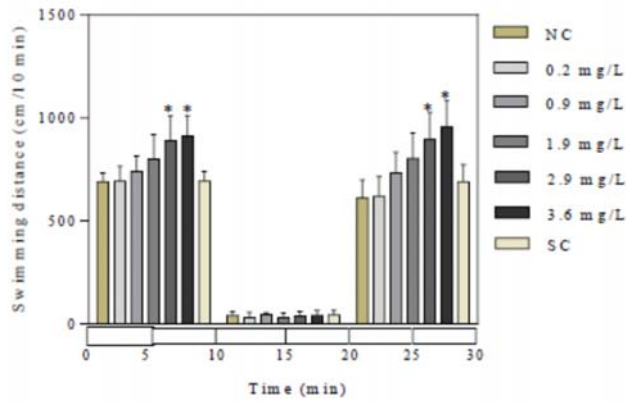


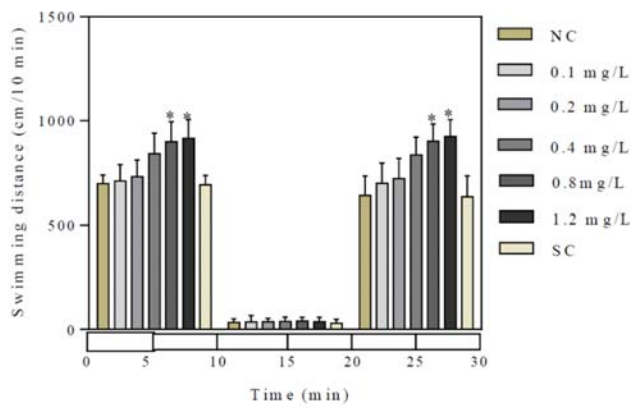
Figure S5: Spontaneous tail coiling's (STC) per x minute after exposure to a) Diazinon, b) Diazoxon and c) Extract

RWTH (Light-dark-transition-test)

A) Diazinon



B) Diazoxon



C) Extract

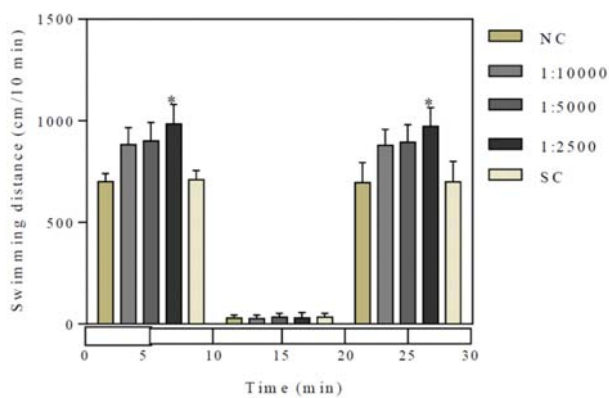


Figure S6: Swimming distance per 10 minutes for the light-dark-transition test (dark-light-dark phase).

VU

Light-dark-transition-test

Diazinon

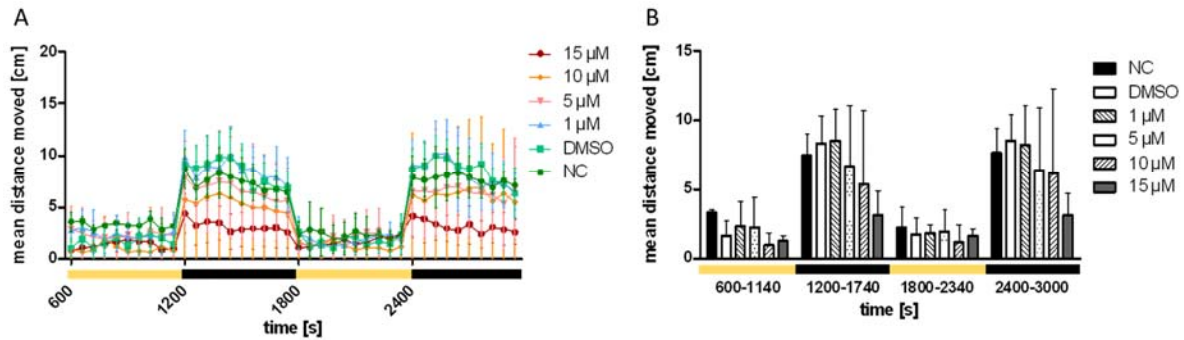


Figure S7: (A) Mean distance moved in cm of embryos exposed to Diazinon. (B) mean distance moved in cm of embryos exposed to Diazinon per 10 minutes. Error bars represent the standard error of mean (SEM), yellow and black bars represent light and dark periods. Statistically significant or highly significant results compared to solvent control (DMSO) are marked by * ($p < 0.05$); ** ($p < 0.01$); or *** ($p < 0.001$). NC= negative control, DMSO= solvent control of 0.1% DMSO.

Diazoxon

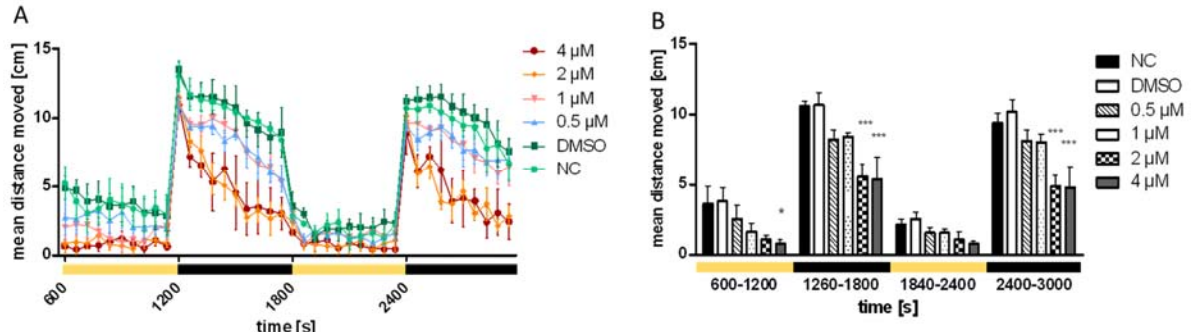


Figure S8: (A) Mean distance moved in cm of embryos exposed to Diazoxon. (B) mean distance moved in cm of embryos exposed to Diazoxon per 10 minutes. Error bars represent the standard error of mean (SEM), yellow and black bars represent light and dark periods. Statistically significant or highly significant results compared to solvent control (DMSO) are marked by * ($p < 0.05$); ** ($p < 0.01$); or *** ($p < 0.001$). NC= negative control, DMSO= solvent control of 0.1% DMSO.

Extract

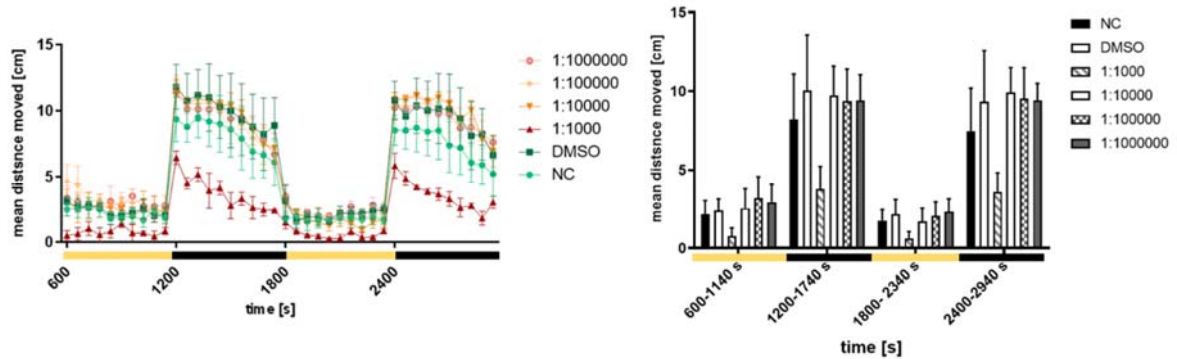


Figure S9: (A) Mean distance moved in cm of embryos exposed to the Extract. (B) mean distance moved in cm of embryos exposed to the Extract per 10 minutes. Error bars represent the standard error of mean (SEM), yellow and black bars represent light and dark periods. Statistically significant or highly significant results compared to solvent control (DMSO) are marked by * ($p < 0.05$); ** ($p < 0.01$); or *** ($p < 0.001$). NC= negative control, DMSO= solvent control of 0.1% DMSO.

Locomotion test

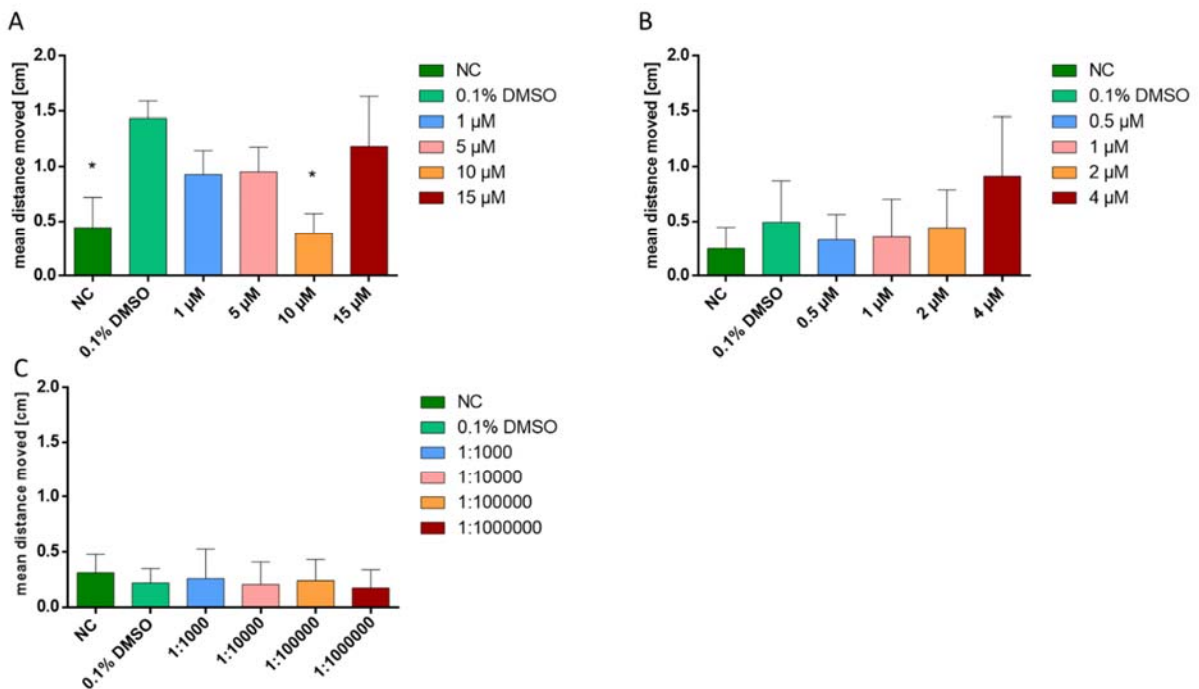


Figure S10: mean distance moved in cm of embryos during continuous light (10 minutes) with embryos exposed to (A) Diazinon, (B) Diazoxon and (C) the Extract. Error bars represent the standard deviation

(SD). Statistically significant results compared to solvent control (DMSO) are marked by * ($p < 0.05$); ** ($p < 0.01$); or *** ($p < 0.001$). NC= negative control, 0.1% DMSO= solvent control.

Touch Response test

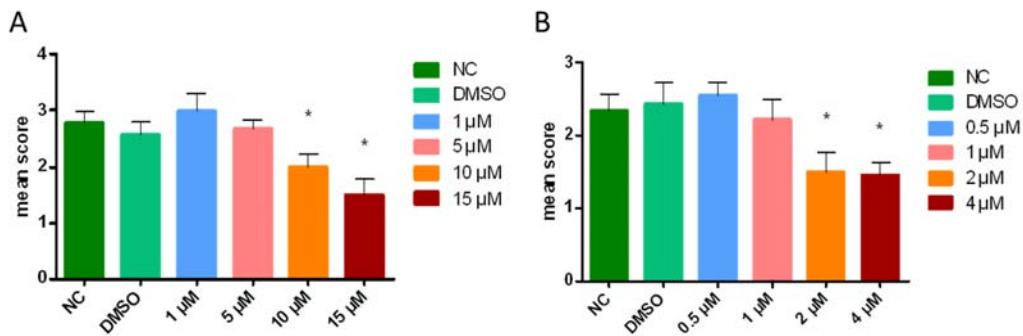


Figure S11: mean scores of embryos in the touch response assay with embryos exposed to (A) Diazinon, (B) Diazoxon. Error bars represent the standard deviation (SD). Statistically significant results compared to solvent control (DMSO) are marked by * ($p < 0.05$); ** ($p < 0.01$); or *** ($p < 0.001$). NC= negative control, DMSO= solvent control of 0.1% DMSO

PMR

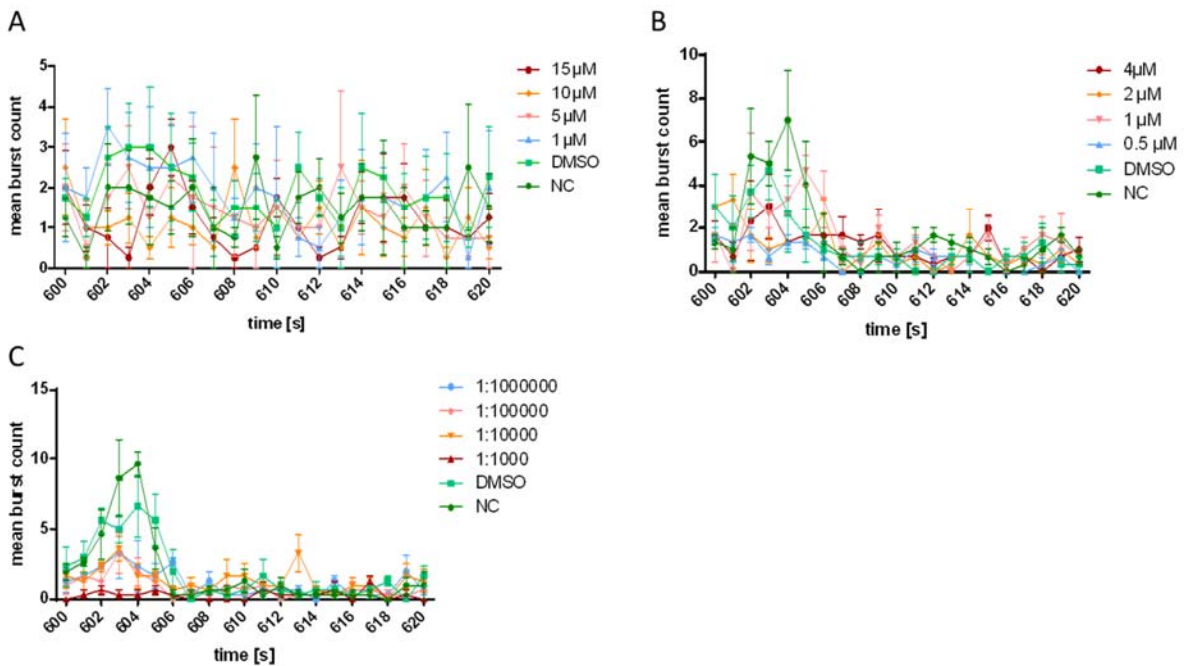


Figure S12: (A) Mean burst counts after the light flash at second 600 of embryos exposed to Diazinon(A), Diazoxon (B) and the Extract (C) in a time course. Error bars represent the standard error of mean (SEM). Differences for 1:1000 are due to high mortality in this concentration. NC= negative control, DMSO= solvent control of 0.1% DMSO

Spontaneous movement test

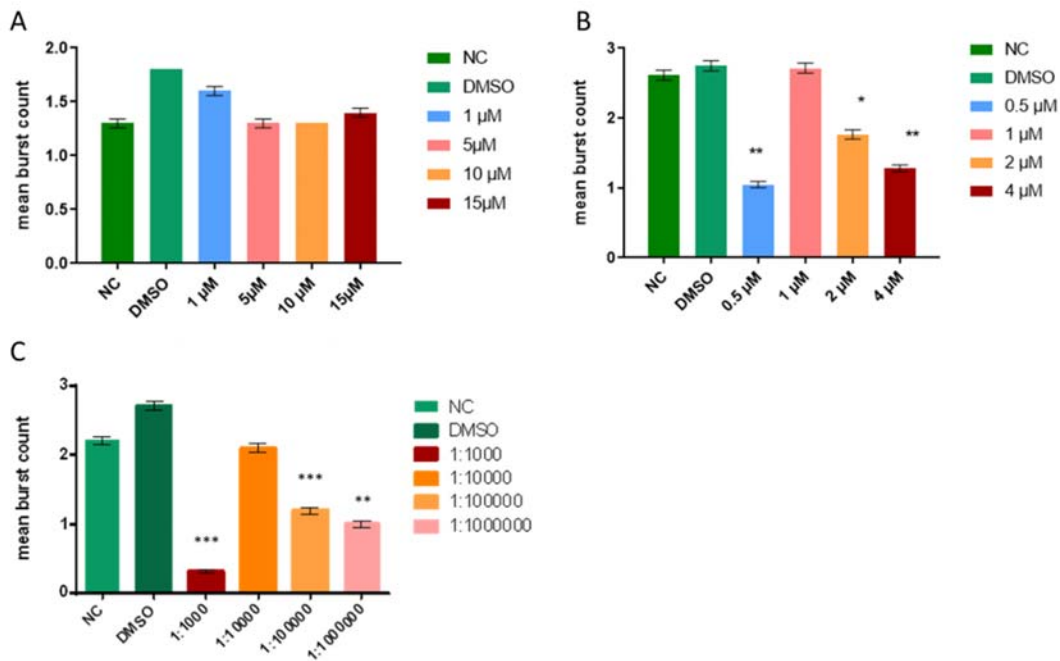


Figure S12: Mean burst counts during 10 minutes' spontaneous movement assay of embryos exposed to Diazinon(A), Diazoxon (B) and the Extract (C). Error bars represent the standard deviation (SD). Statistically significant or highly significant results compared to solvent control (DMSO) are marked by * ($p < 0.05$); ** ($p < 0.01$); or *** ($p < 0.001$). Significant differences for 1:1000 are due to high mortality in this concentration. NC= negative control, DMSO= solvent control of 0.1% DMSO.

MTM

Tapping test

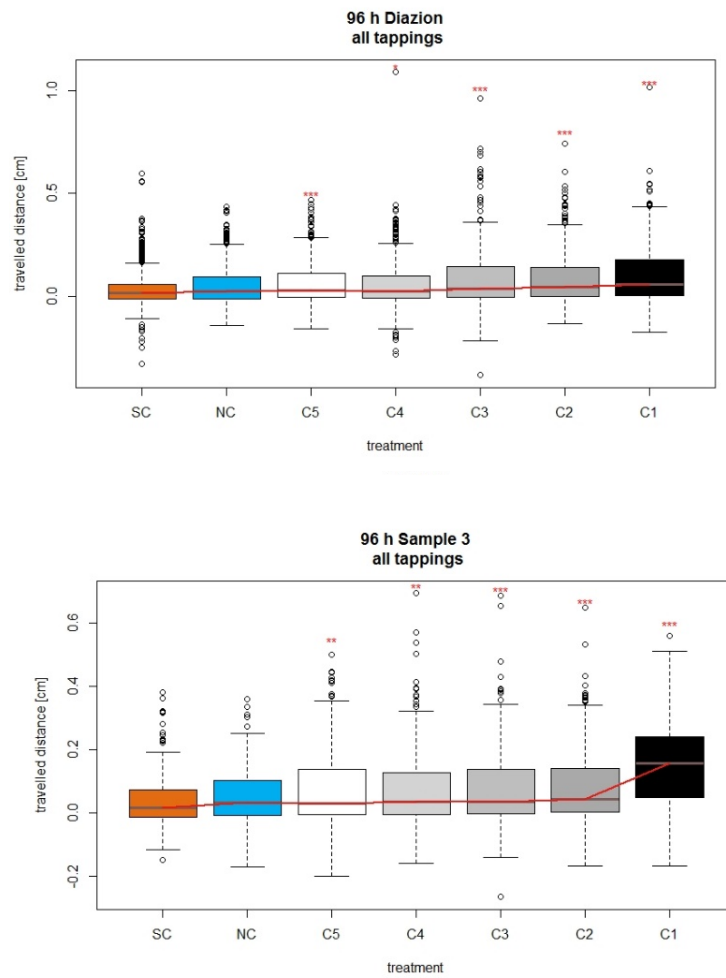


Figure S13: Boxplots of the travelled distance in cm after 96 h exposure to Diazion, Diazoxon and the Extract (sample 3) under light conditions. SC= Solvent control; NC= Negative control; C5- C1= Exposure dilution series. The red line connects the median values from each treatment (each Boxplot). Asterisks indicate the different significance levels: $p < 0.05 = *$; $p < 0.01 = **$; $p < 0.001 = ***$; Blacklined circles show single values.