Investigation of parathion-induced neurotoxicity in SHSY5Y cells demonstrates utility of the assay as an alternative animal model

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Acknowledgment

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Problem:

- Parathion is an organophosphorus (OP) pesticide used worldwide which exhibits the characteristic OP mechanism of toxicity, the inhibition of acetylcholinesterase causing hyper-excitation of the cholinergic nervous system.

in vitro Approach for Toxicity Investigations

- Given that environmental release of parathion-based pesticides is the standard use case, we were interested in characterizing parathion and sunlight-transformed parathion toxicity in human neuronal cell line (SHSY5Y) and in cell free assays.
- The neuronal cell-based assays were used to determine impacts of parathion on cell viability, formation of reactive oxygen species (ROS), cell membrane peroxidation, mitochondrial membrane potential and apoptosis.

Cell-Free Assay - Novel Environmental Toxicity

- Sunlight-induced degradation of ethyl-parathion and cell-free screening were used to assess reactions with DNA and linoleic acid.

Methods: *in vitro* Assays

- A range finding test was used to determine effective concentrations of ethyl-parathion on SHSYSY cell viability by MTT assay (Fig. 1). Test concentrations 0, 5 & 10 µg/ml were selected for all cell assays.
- Intracellular ROS was determined by 2',7'-dichlorofluorescein diacetate (DCFDA) assay.
- Oxidative damage to lipid in neuronal cells SHSH5Y was determined by trichloroacetic acid (TCA) methods with absorbance at 532nm.
- Mitochondrial membrane potential was determined by JC-1 dye.
- Immunofluorescence of Annexin V was used to assess apoptosis.

Methods: Cell-Free Assays

- Sunlight-light degradation of ethyl-parathion occurred at average ultra-violet A (UVA) at 7.15 mW/cm^2.
- ROS was measured photochemically (absorbance at 440nm) in the cell-free parathion sunlight-degradation assays was & confirmed by ultra performance liquid chromatography.
- Degradation of DNA (guanine base, 2dGuO) recorded at 260 nm and linoleic acid photoperoxidation recorded at 233nm was also measured in response to sunlight-degraded parathion.

Human neuronal cell-based assay

- Ethyl-parathion significantly reduced neuronal cell (SHSY5Y) viability (Fig. 1)

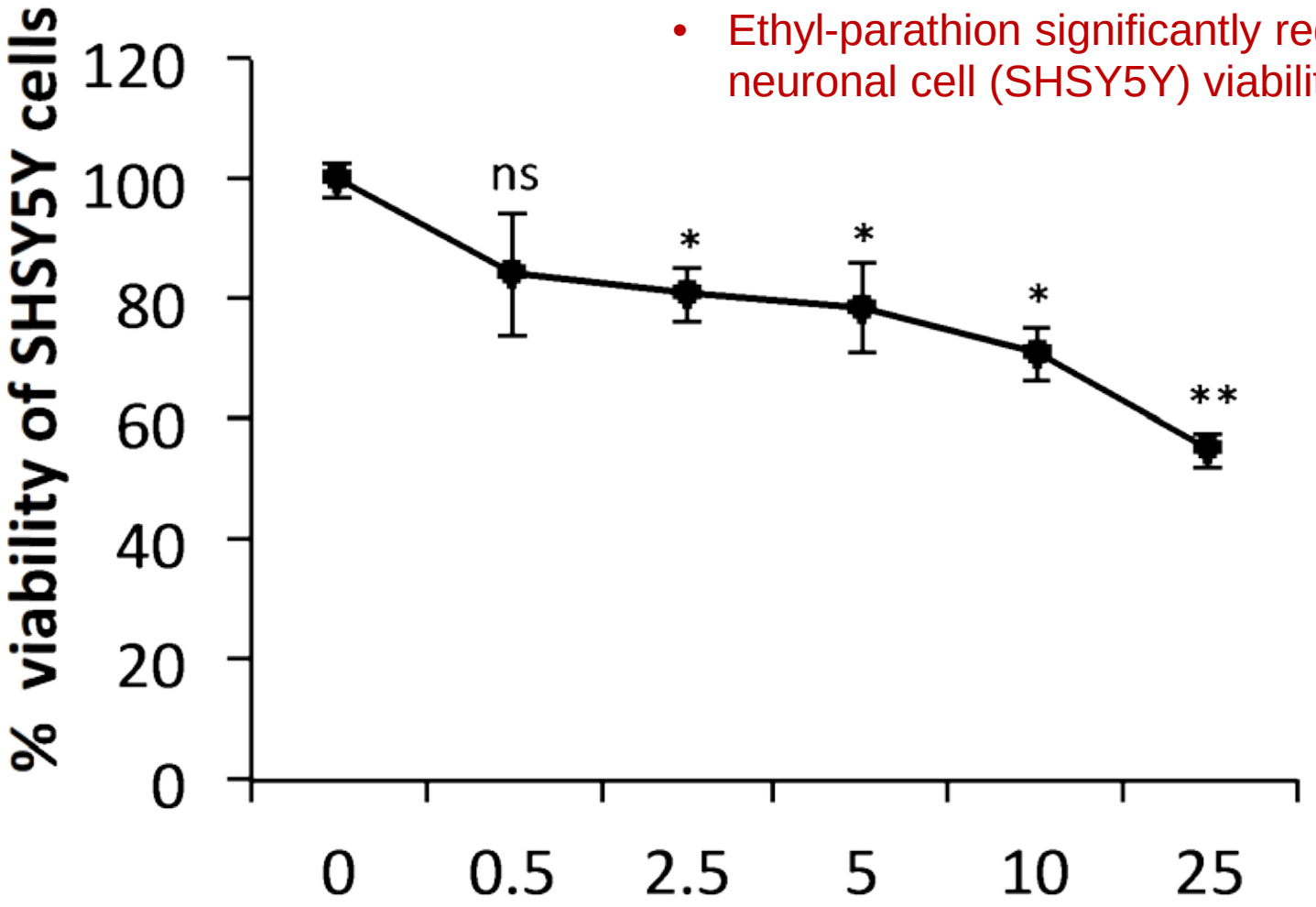


Fig 1: Viability of human neuronal cells SHSY5Y with different concentration of ethyl parathion by MTT assay. Formazan absorbance was measured at 570 nm using Biotek microplate reader. % viability was calculated with respect to viability of cells with 0 µg/ml of ethyl parathion.

Human neuronal cell-based assay

- Effective doses of Ethyl-Parathion caused significant increases in ROS generation (Fig. 2)

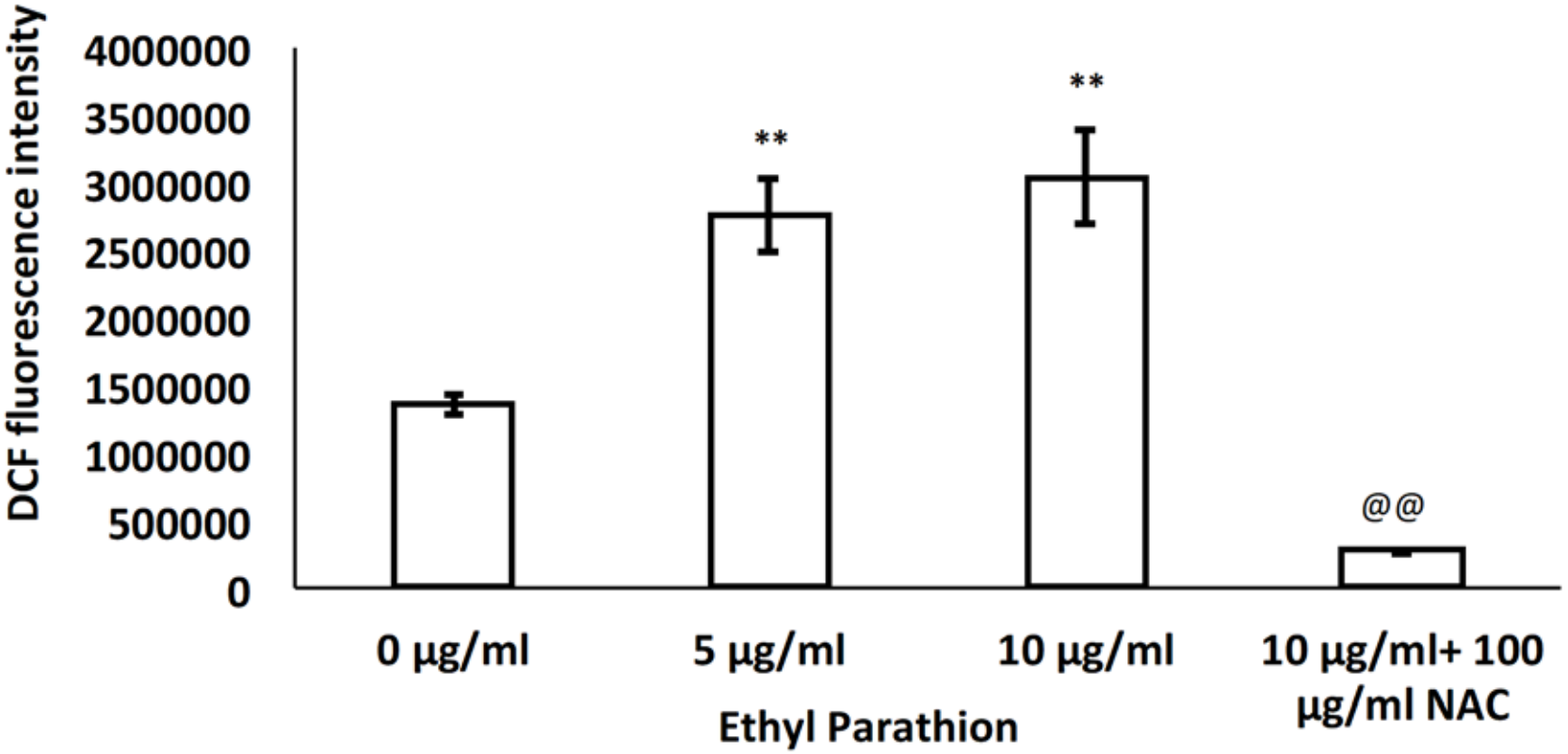
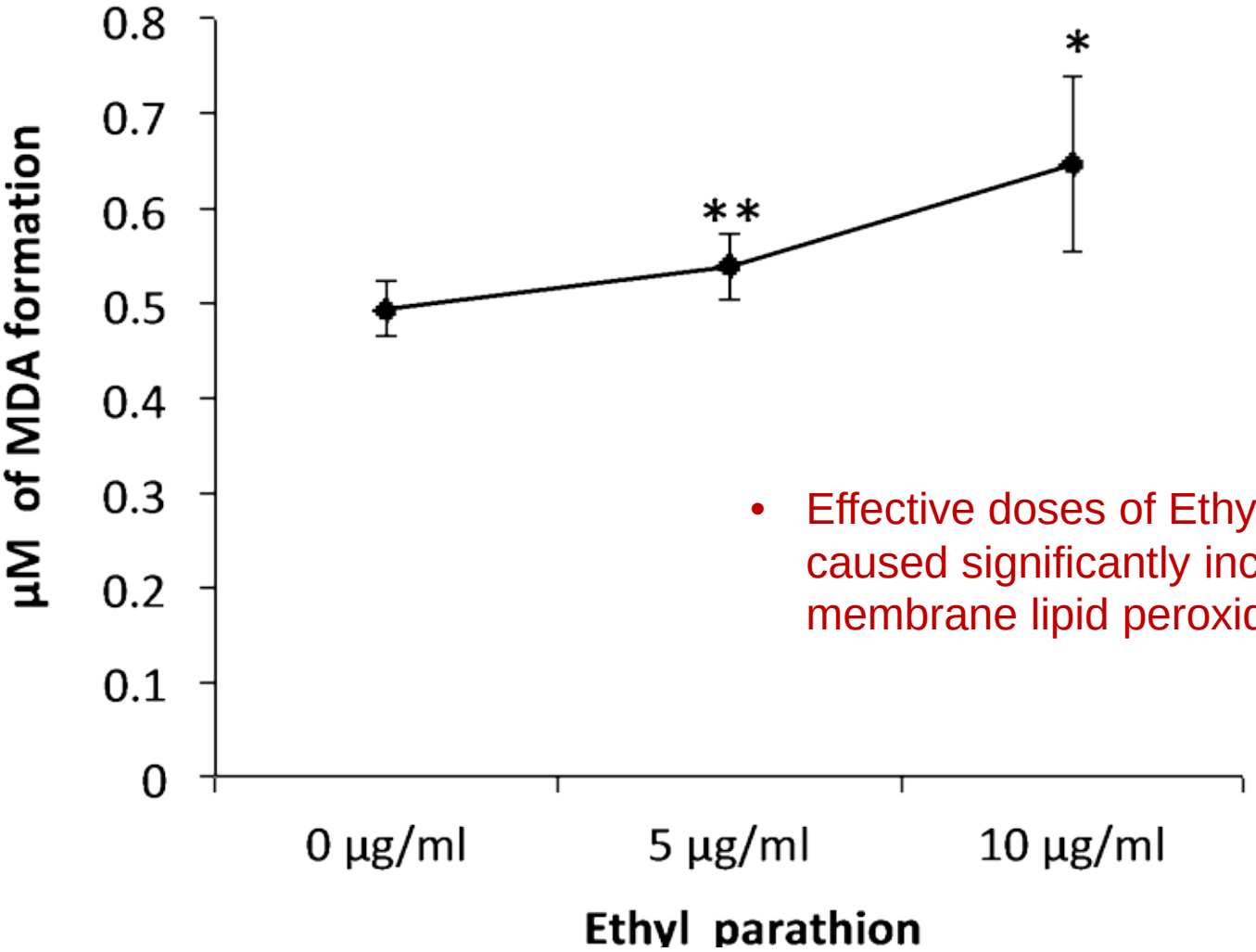


Fig 2: DCF fluorescence intensity induced by ethyl parathion (5 & 10 µg/ml) in SHSY-5Y cells was recorded by microplate reader with excitation 480nm/emission 530nm

Human neuronal cell based assay



- Effective doses of Ethyl-Parathion also caused significantly increased membrane lipid peroxidation (Fig. 3).

Fig 3: Parathion induced lipid peroxidation in SHSY5Y cells. Melondialdehyde (MDA) as end product of lipid membrane peroxidation was estimated at 532 nm using microplate reader.

Human neuronal cell-based assay

- The increased ROS & membrane-lipid peroxidation responses to ethyl-parathion exposure have logical connects to observed loss of mitochondrial membrane potential (Fig. 4)

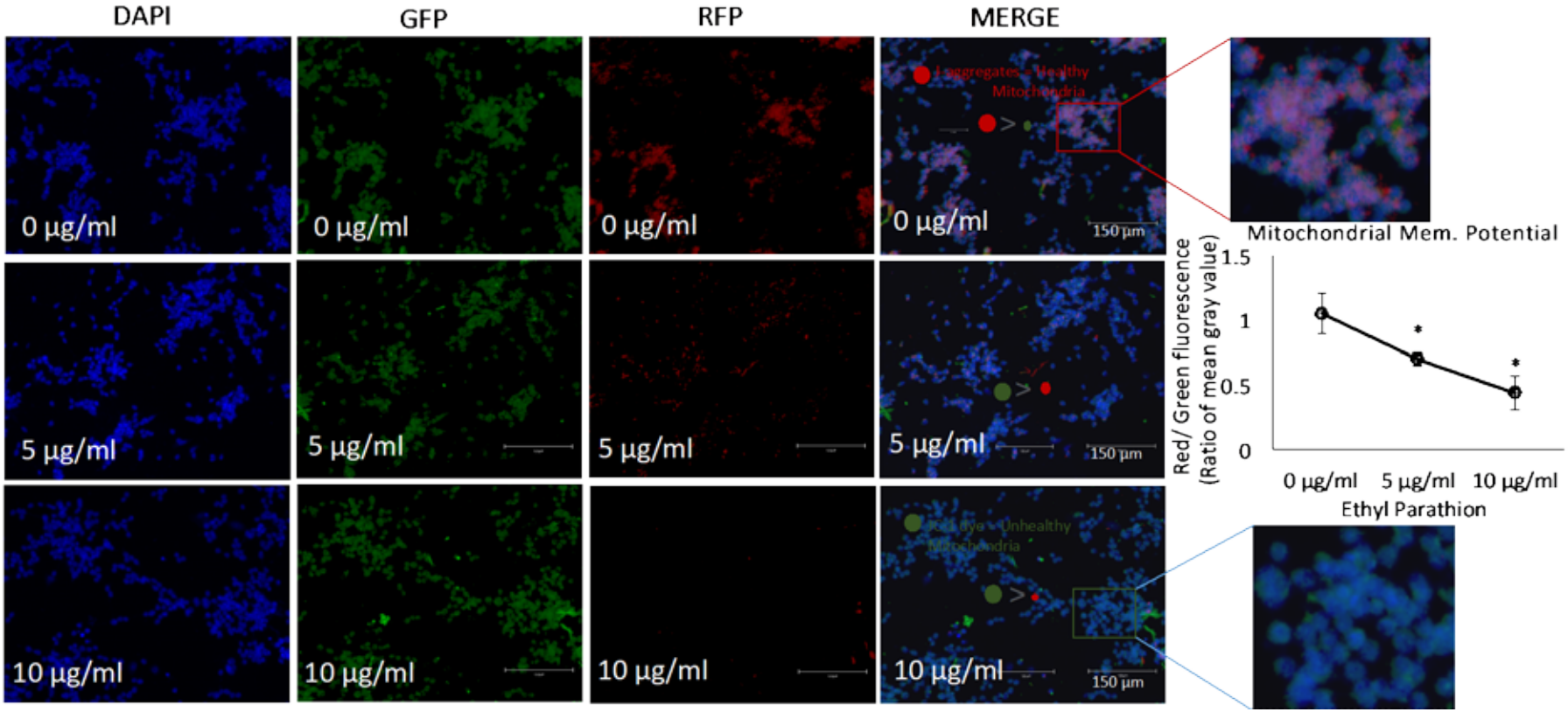


Fig 4: JC-1 staining pattern indicate loss of mitochondrial membrane potential in SHSH5Y cells with exposure of 5 & 10 µg/ml of ethyl parathion. Examined under cell imaging system EVOS 5000. Mean gray value of four replicates were calculated after background subtraction with ImageJ.

Human neuronal cell-based assay

- Finally, increased incidence of apoptosis in the neuronal cells was also observed in response to ethyl-parathion exposure (Fig 5).

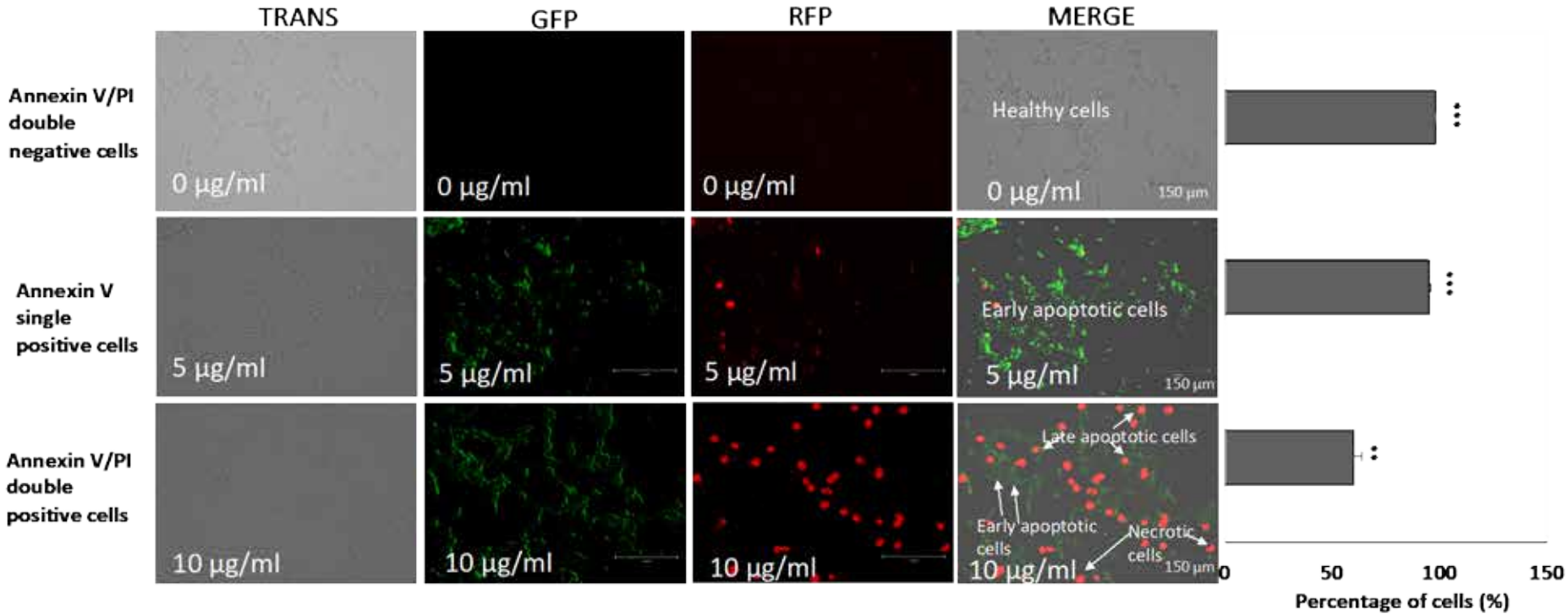


Fig 5: Immunofluorescence image of SHSY5Y cells stained with Annexin V- FITC/PI after treatment with 0, 5 & 10 µg/ml of ethyl parathion. Examined under cell imaging system EVOS 5000. Horizontal bar represents mean of four replicates

Cell-free assays

- Ethyl-parathion was rapidly transformed in sunlight where formation of singlet oxygen species increased with increasing sunlight exposure duration (Fig. 6a).

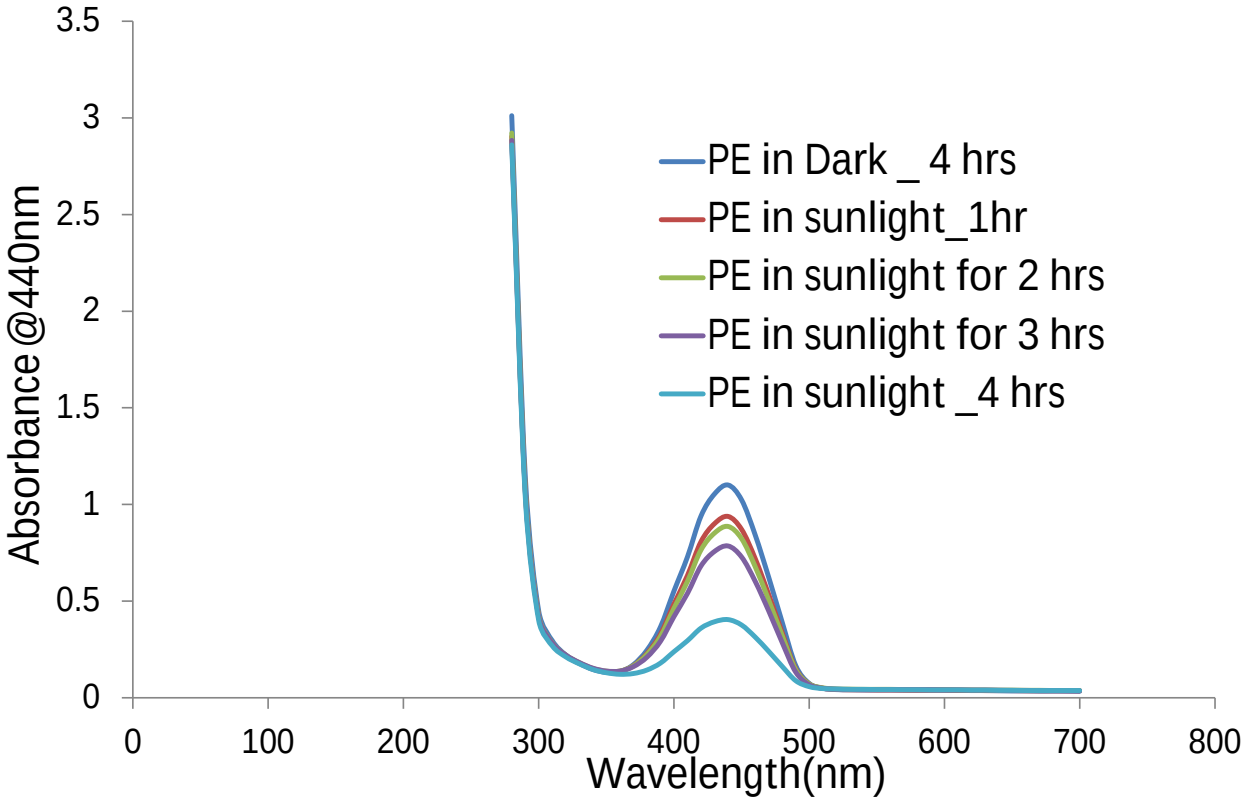


Fig 6(a): Ethyl parathion induced generation of singlet oxygen species in sunlight having ambient intensity of UVA (PE= Parathion ethyl) in buffer, in which photochemical generation of singlet oxygen species was recorded at 440nm.

Cell-free assays

- The ethyl-parathion degradation in sunlight was confirmed by Ultra-performance liquid chromatography (Fig. 6b).

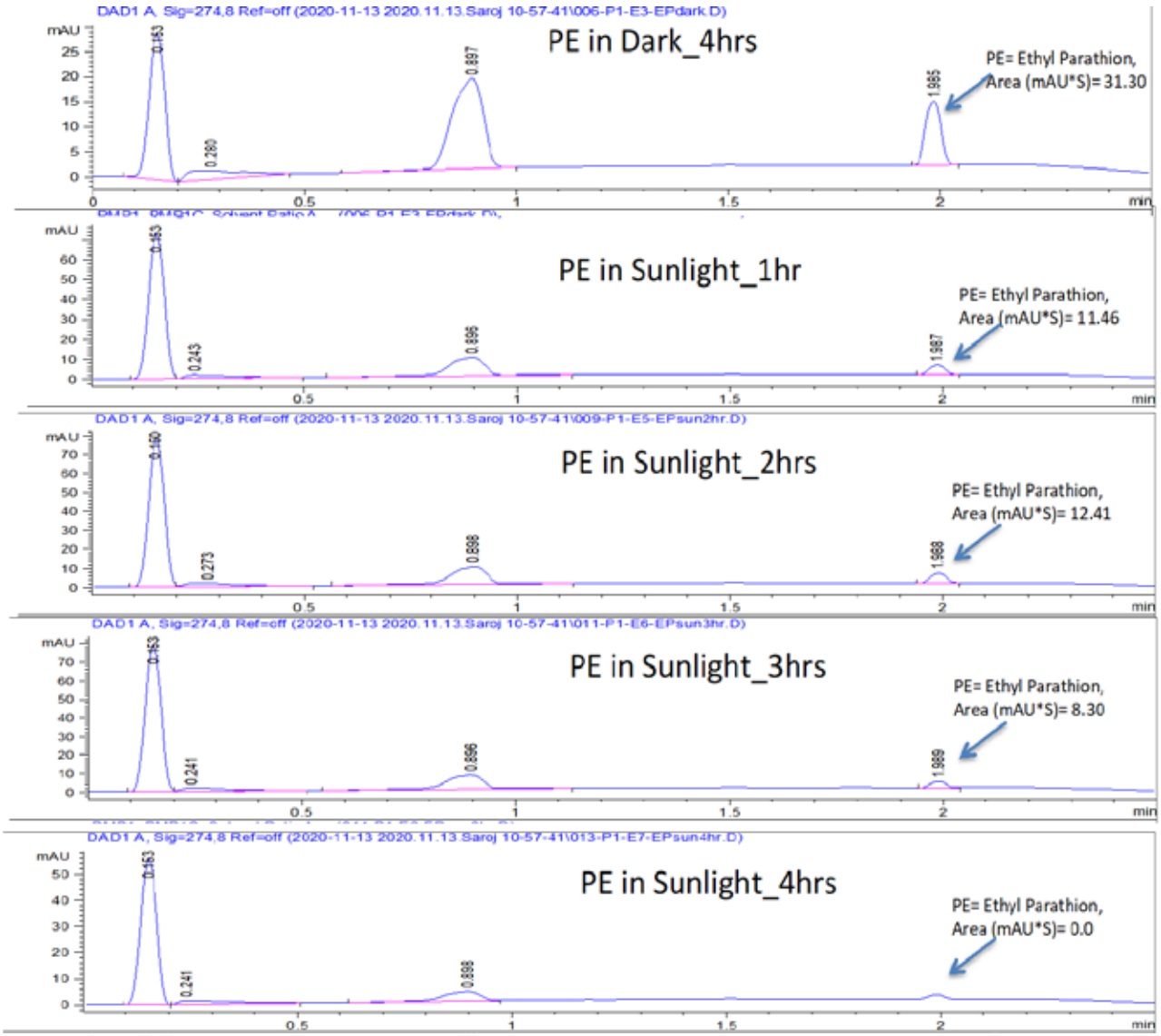


Fig 6(b). Ultra-performance liquid chromatography report confirmed degradation of parathion leads to generation of singlet oxygen species

Cell-free assays

- Cell-free assays indicated increased potential for DNA degradation (Fig. 7.)

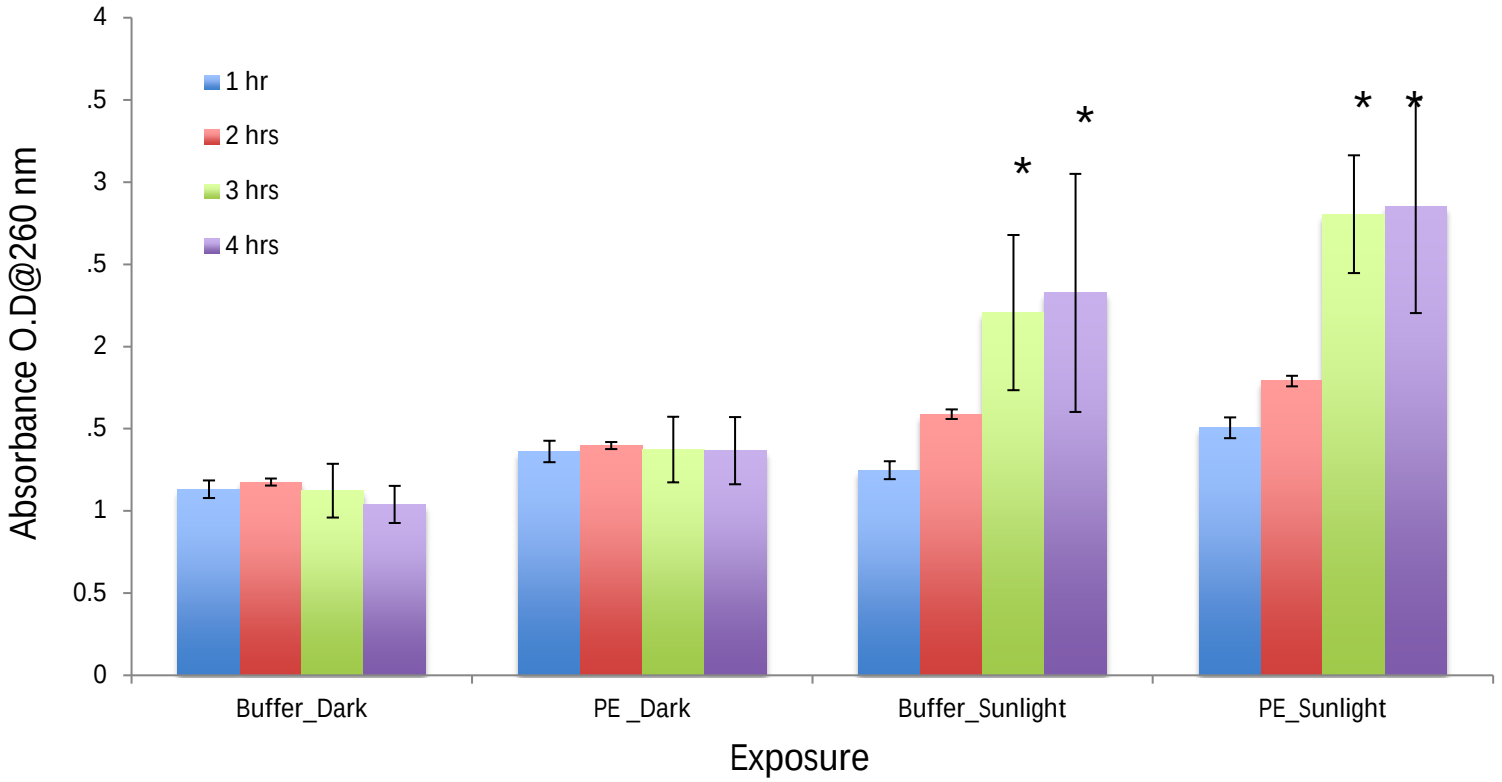


Fig 7. Effect of ethyl-parathion exposure to sunlight (1-4 hours) on DNA degradation (guanine base 2dGuO) with all treatment including 3 replicates

Cell-free assays

- Increased potential for linoleic-acid peroxidation when ethyl-parathion is degraded in sunlight (Fig. 8.).

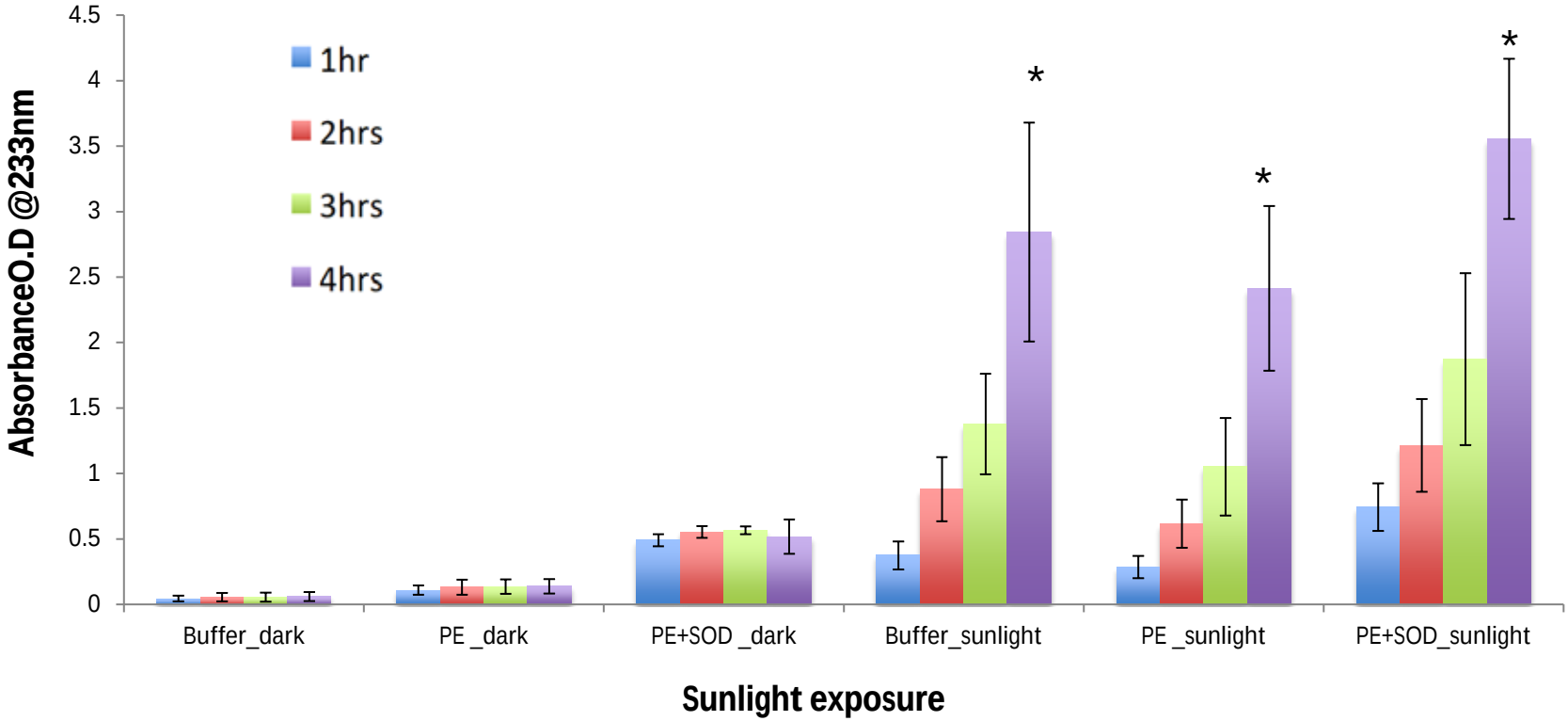


Fig 8: Effect of ethyl-parathion (PE) exposure to sunlight (1-4 hours) on ROS-mediated linoleic-acid peroxidation with all treatments including 3 replicates SOD = Superoxide-dismutase 100Unit).

Study Conclusions

- Ethyl-parathion exposures in the neuronal cell line (SHSY5Y) caused significantly decreased cell viability which was likely the result of decreased mitochondrial membrane potential and increased apoptosis.
- Molecular assays in the neuronal cells indicated mechanisms underlying these responses were likely ethyl-parathion induced ROS production causing membrane lipid peroxidation.
- Cell-free screening suggested sunlight-induced degradation of ethyl-parathion poses novel environmental risks of singlet oxygen formation which can cause damage to fundamental DNA and cell membrane building blocks.

Value of the *in vitro* & cell-free assays

- Results of these non-animal tests provided mechanistic toxicology outcomes that parallel responses observed in *in vivo* tests.
- These methods show great potential for mechanistic toxicology screening of neurotoxic chemicals and should be considered for early tier hazard characterizations.


Thanks.