

**[P-24]****Tributyltin induce apoptosis by disturbance of Ca<sup>2+</sup> and mitochondrial activity, causing oxidative stress and activation of caspases in R2C**

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Tributyltin (TBT) used world-wide in antifouling paints for ships is a widespread environmental pollutant and cause reproductive organs atrophy in rodents. At low doses, antiproliferative modes of action have been shown to be involved, whereas at higher doses apoptosis seems to be the mechanism of toxicity in reproductive organs by TBT. In this study, we investigated that the mechanisms underlying DNA fragmentation induced by TBT in the rat leydig cell line, R2C. TBT was assayed intracellular Ca<sup>2+</sup> level and intracellular reactive oxygen species (ROS) in R2C by fluorescence detector. As the results, TBT induced to significantly increase intracellular Ca<sup>2+</sup> level in a dose- and time-dependent manner. The rise in intracellular Ca<sup>2+</sup> level was followed by a dose- and time-dependent generation of reactive oxygen species (ROS) at the mitochondrial level. Simultaneously, TBT induced the release of cytochrome c from the mitochondrial membrane into the cytosol. Furthermore, ROS production and the release of cytochrome c were reduced by BAPTA, an intracellular Ca<sup>2+</sup> chelator, indicating the important role of Ca<sup>2+</sup> in R2C during these early intracellular events. In addition, that Z-DEVD FMK (mainly a caspase-3 inhibitor) decreased

apoptosis by TBT. Taken together these data show the apoptotic pathway followed by TBT starts with an increase of intracellular  $\text{Ca}^{2+}$  level then continues with release of ROS and cytochrome c from mitochondria, activation of caspases, and finally results in DNA fragmentation.

**Key word** : tributyltin, reactive oxygen species, intracellular  $\text{Ca}^{2+}$ , caspase activity