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[Lab. of Public Health]

Investigation of the Hemolytic Effects of Various Organophosphoric Acid Triesters (OPEs) and their Structure-activity Relationship.

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Organophosphoric acid triester (OPEs) have been widely used in industrial and consumer products as fire retardants, plasticizers and high temperature functional fluids. The hemolytic effects of various OPEs were investigated and they showed strong hemolytic toxicity except triethyl phosphate and tris(chloroethyl)phosphate. 2-Ethylhexyldiphenyl phosphate (EHDP) showed the strongest toxicity. Quantitative structure-activity relationship (QSAR) study was performed using various physicochemical and topological parameters. One-parameter regression equation for enough to estimate hemolysis was not obtained. But, the high enough two parameter regression equations were obtained for estimating EC_{50} and EC_{20} . The correlation coefficients were 0.943 for $\log(1/EC_{50})$ and 0.973 for $\log(1/EC_{20})$ using n-octanol/water coefficient ($\log P$) and molecular connectivity index $4 \chi_p^v$.

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[Lab. of Public Health]

Relationship between Hemolytic Toxicity and Signal Intensity of Various Organotin Compounds by a Spin-labeling Technique.

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Various organotin compounds act as environmental pollutants and we demonstrated that tri-n-butyltins and triphenyltins have higher hemolytic activities than sodium-n-dodecyl sulfate (SDS). The hemolysis may occur by damage to the biomembrance of erythrocytes. Spin-labeling techniques with electron spin resonance (ESR) spectroscopy is a useful technique for investigation of the nature of biomembrances. The relationship between hemolytic toxicity and signal intensity was investigated. Two kinds of spin-labeled stearic acid in which the paramagnetic center was located at different sites on the alkyl chain (5- and 12-doxy-stearic acids, 5- and 12-NS) were used. The shape of the ESR signal did not change with the organotin compounds and only the peak height decreased, and it became clear that the decrease of ESR signal intensity was related to the hemolytic toxicity of the organotin compounds. We observed that the decrease in signal intensity for 12-NS was generally larger than those for 5-NS.

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[Lab. of Public Health]

Studies on Free Radical Scavenging Activities of Natural Materials

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Antioxidant activities of natural materials were examined with ESR-spin trapping method. For natural materials, propolis, bee products etc. were tested. Propolis is a resinous hive product, collected by honey-bees and is known for its anti-inflammatory activities etc. The components and biological activities depends on the geographic origin. The propolis we used were from Denmark (water soluble), China, Brazil, Uruguay, New Zealand. For hydroxyl radical, propolis from Denmark, and low allergenic one from China had high scavenging activity. For DPPH radical, ones from New Zealand, China and low allergenic one from China had high activity. For O_2^- , ones from Brazil, China and New Zealand had strong activity. For in vivo experiment, inhibitory effect of natural materials to the induction of chromosomal aberration by MMC were examined with in vivo micronucleus method. Inhibitory effects were found in propolis from Brazil and Uruguay, remarkably. So, these activity seems to related to the O_2^- scavenging activity.

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[Lab. of Biochemistry]

Switch of Coenzyme Specificity of Mouse Lung Carbonyl Reductase by Substitution of Threonine 38 with Aspartic Acid.

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Mouse lung carbonyl reductase (MLCR), a member of the short-chain dehydrogenase/reductase (SDR) family, exhibits coenzyme specificity for NADP(H) over NAD(H). Crystal structure of the enzyme-NADPH complex shows that Thr-38 interacts with the 2'-phosphate of NADPH and occupies the position spatially similar to an Asp residues of the NAD(H)-dependent SDRs that hydrogenbonds to hydroxy groups of the adenine ribose of the coenzymes. The mutant (Thr-38 replaced with Asp) resulted in increases of more than 200-folds in the K_m values for NADP(H) and decreases of more than 7-folds in those for NAD(H). These results indicate a significant role of Thr-38 in NADP(H) binding for MLCR and provide further evidence for the key role of Asp at this position in NAD(H) specificity of the SDR family proteins.