The Rapid Detection of Toxic Compounds by New Cytotoxicity Test

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Well–known toxic compounds contained in fishes, shellfishes and plants have been detected with chemical and physical methods. However, it is expected that the determination of the total toxicity with the above methods is very difficult because water is contaminated with the various unknown and known compounds. The caluculation of total toxicity is impossible even if all of the toxic compounds was determined.

On the other hand, animals have been used for the determination of total toxic events, but the animal testing requires many animals and long time for the determination of the toxicity level.

We propose the rapid and accurate detection of toxicity with mammalian cells. The proposed assay with mammalian cells require only 10–20 min and is much faster than usual cytotoxicity tests such as neutral red inclusion and MTT (3–(4,5–dimethylthiazol–2–yl)–2,5–diphenyltetrazolium bromide) reduction assays which require 3–5 h.

The principle of the proposed assay is as follow .
The viable cells have NAD(P)H:menadione oxidoreductase in plasma membrane other than cytosol, mitochondria and microsomes, and exogenous menadione catalyzes reduction of dissolved oxygen to hydrogen peroxicide by reacting with NAD(P)H:menadione oxidoreductase in plasma membrane of viable cells supplying NAD(P)H. When menadione is added to the cell culture, menadione–catalyzed H_2O_2 production by viable cells is proportional to viable cell number. The incubation time for the production of H_2O_2 is only 5–10 min. The concentration of H_2O_2 produced is determined by chemiluminescent assay for 5 seconds or by colorimetrical assay for 5 min. Clear cytotoxic effects are observed by the proposed assay rather than neutral red inclusion and MTT reduction assays.

The proposed assay is able to detect the cytotoxicity of water, foods, drugs and other materials after 1 h–exposure of cells to toxic compounds. Therefore, the proposed assay is expected to be useful for the rapid detection of toxicity of water, plants, fishes and shellfishes contaminated by various toxic compounds in coastal seas.

Reference: Analytical Biochemistry 207, 255–260 (1992)