

Application to halogenized propanes of Simple and Rapid Sperm Toxicity Tests by Tetrazolium Salt Methods in Rats

*Katsumi Ohtani**, *Kenichi Kobayashi*, *Hisayo Kubota*, and *Junzo Saegusa*

National Institute of Industrial Health, Independent Administrative Institution, Japan

[Introduction] Spermatotoxicity of Dibromochloropropane (DBCP) or 2-bromopropane (2BP) was evidenced by incidents of workplace exposure in foreign countries. This finding suggested the importance of testicular toxicity testing in the industrial toxicology. It has also been recognized since the issue of endocrine disrupters arose all over the world. Manual (microscopic) observation is, however, still used widely in sperm quality testing, and thus development of more reliable and objective methods is needed. We examined the applicability of six tetrazolium (MTT/WST-1/-3/-8/MTS/XTT) assays to the assessment of sperm quality as a simple, rapid and objective instrumental method, comparing these assays with other instrumental methods, such as the uses of Sperm Quality Analyzer (SQA) and Computer-Assisted Sperm Analyzer (CASA; HTM-IVOS).

[Methods] The epididymal fluid derived from male F344 rats treated subcutaneously with DBCP, 2BP, 1-bromopropane (1BP) or 1,2-dichloropropane (DCP) twice a week, totally 8 times, was examined by all these assays.

[Results] Significant changes in absorbance levels by MTT and WST-3 assays were observed in the high dose of DBCP, 1BP and 2BP treated groups as well as in the Sperm Count by CASA, while no significant difference was evident only in WST-1 assay. Furthermore, significant decreases in the sperm motility indices obtained by SQA and CASA were observed in the similar DBCP and 1BP groups, while no such decrease was observed in all the 2BP treated groups. As concerned with DCP treated groups, no significant difference was observed in all the assays except WST-8 assay.

[Discussion] These results indicate that most tetrazolium (MTT, WST-3, MTS, XTT) assays can detect sperm toxicity caused by introduced chemical agents to the comparable extent as other methods such as CASA or SQA method. While the formazan formed by MTT is insoluble in water, WST-3 generates water-soluble one therefore the step of dissolving formazan prior to spectrometry is unnecessary in WST-3 assay. The MTT and WST-3 assays proved to be more sensitive than other tetrazolium salt assays. MTS, XTT and WST-3 have advantage of forming water-soluble formazan making procedure simpler. Accordingly, WST-3 is thought to be the best salt for the tetrazolium method of sperm analysis.

[Acknowledgement] This study was partly supported by the Special Coordination Funds for Promoting Science and Technology of the Science and Technology Agency of the Japanese Government.