（学位第３号様式）

学 位 論 文 要 旨

氏　名	Kazunori Narumi
題　目	Developmental Study of Repeated-Dose Liver Micronucleus Assay Using Adult Rats （成熟ラットを用いた反復投与肝小核試験法の開発に関する研究）

Liver micronucleus (MN) assays, which detect chromosomal damage by measuring the induction of micronucleus formation, are highly sensitive in predicting hepatocarcinogenicity and useful in evaluating genotoxic substances that target the liver. The animal welfare movement, which emphasizes the "3Rs" (reduction, replacement, and refinement of animal use), have recently focused on regulatory toxicity testing, requiring integration of genotoxicity endpoints into repeated-dose toxicity (RDT) studies. However, the previously developed methods require artificial mitotic stimulation by partial hepatectomy or mitogen treatment or the use of young rat liver to compensate for the low mitotic activity of adult rat hepatocytes. These methods are also typically performed using *in situ* perfusion of the whole liver to isolate hepatocytes. For both of these reasons, the liver MN assay methods could not be integrated into general RDT studies which utilize intact adult animals.

In this study, I focused on the long life span (at least 200 days) and slow turnover of adult rat liver hepatocytes. At first, to confirm whether repeated administration of hepatocarcinogens causes accumulation of micronucleated hepatocytes (MNHEPs) in the liver, diethylnitrosamine (DEN) and 2, 4-diaminotoluene (2, 4-DAT) were repeatedly orally administered to adult male rats for 5, 14, and 28 days. Induction of MNHEPs was achieved after administration of DEN for 5 days or longer and that of 2, 4-DAT for 14 days or longer, and the induction levels were increased depending on the number of administrations. Additionally, to enable the liver tissue examined concurrently with other toxicological endpoints, alternatives to the *in situ* perfusion method were examined. The established method of hepatocyte isolation is quite easy, involving only treatment of a portion of the liver by using collagenase in a centrifuge tube.

Moreover, to directly observe hepatocyte fate after micronucleus formation, DEN was repeatedly administered orally to adult male rats for up to 2 weeks and the thymidine analog 5-ethynyl-2'-deoxyuridine (EdU) was injected intraperitoneally on day 1, 7, or 14. Hepatocytes were isolated at 24 h, 1 week, or 2 weeks after EdU injection. MNHEPs labeled using EdU on the first day persisted until 2 weeks post-administration in the livers. However, the frequency of MHNEPs among EdU-labeled hepatocytes decreased over time. Additionally, the number of TUNEL-positive cells in the liver tissue increased, suggesting selective removal of micronucleated cells. Theoretical calculation of the cumulative MNHEP frequency on each of the days on which DEN was administered, taking into account the rate of loss, came out closer to the actual value observed in the liver MN test.

These results suggested that (1) the established method for hepatocyte isolation without using the *in situ* perfusion technique enables the integration of liver micronucleus assays into general toxicity studies; (2) hepatocytes micronucleated by a genotoxic hepatocarcinogen may undergo selective removal; nevertheless, they persist for a long period in a certain proportion, and repeated administration results in their accumulation and increased frequency; (3) even in adult rat liver tissues, micronucleus induction is detectable with high sensitivity by administering repeated doses of the test compound for 14 days or more.

The repeated-dose liver MN assay developed in this study will reduce the number of animal experiments, and provide a method for comprehensive evaluation of toxicological risks of a test compound over a relatively low exposure range.