



Standard Practice for *In Vivo* Rat Hepatocyte DNA Repair Assay¹

This standard is issued under the fixed designation E 1398; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers a typical procedure and guidelines for conducting the rat *in vivo* hepatocyte DNA repair assay. The procedures presented here are based on similar protocols that have been shown to be reliable (1, 2, 3, 4, 5).²

1.2 Mention of trade names or commercial products are meant only as examples and not as endorsements. Other suppliers or manufacturers of equivalent products are acceptable.

1.3 *This standard does not purport to address all of the safety concerns associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Significance and Use

2.1 Measurement of chemically induced DNA repair is a means of assessing the ability of a chemical to reach and alter the DNA. DNA repair is an enzymatic process that involves recognition and excision of DNA-chemical adducts, followed by DNA strand polymerization and ligation to restore the original primary structure of the DNA (6). This process can be quantitated by measuring the amount of labeled thymidine incorporated into the nuclear DNA of cells that are not in S-phase and is often called unscheduled DNA synthesis (UDS) (7). Numerous assays have been developed for the measurement of chemically induced DNA repair in various cell lines and primary cell cultures from both rodent and human origin (4). The primary culture rat hepatocyte DNA repair assay has proven to be particularly valuable in assessing the genotoxic activity of chemicals (8). Genotoxic activity often results from metabolites of a chemical. The *in vitro* rat hepatocyte assay provides a system in which a metabolically competent cell is also the target cell. Most other *in vitro* short-term tests for genotoxicity employ a rat liver homogenate (S-9) for metabolic activation, which differs markedly in many important ways

from the patterns of activation and detoxification that actually occur in hepatocytes. An extensive literature is available on the use of *in vitro* DNA repair assays (9-19).

2.2 A further advance was the development of an *in vivo* rat hepatocyte DNA repair assay in which the test chemical is administered to the animal and the resulting DNA repair is assessed in hepatocytes isolated from the treated animal (20). Numerous systems now exist to measure chemically induced DNA repair in specific tissues in the whole animal (4). The average of *in vivo* assays is that they reflect the complex patterns of uptake, distribution, metabolism, detoxification, and excretion that occur in the whole animal. Further, factors such as chronic exposure, sex differences, and different routes of exposure can be studied with these systems. This is illustrated by the potent hepatocarcinogen 2,6-dinitrotoluene (DNT). Metabolic activation of 2,6-DNT involves uptake, metabolism by the liver, excretion into the bile, reduction of the nitro group by gut flora, readsorption, and further metabolism by the liver once again to finally produce the ultimate genotoxicant (21). Thus, 2,6-DNT is negative in the *in vitro* hepatocyte DNA repair assay (22) but is a very potent inducer of DNA repair in the *in vivo* DNA repair assay (23, 24). A problem with tissue-specific assays is that they may fail to detect activity of compounds that produce tumors in other target tissues. For example, no activity is seen in the *in vivo* DNA repair assay with the potent mutagen benzo(a)pyrene (BP), probably because limited tissue distribution and greater detoxification in the liver yields too few DNA adducts to produce a measurable response (3). In contrast, BP is readily detected in the less tissue-specific *in vitro* hepatocyte DNA repair assay (11). An extensive literature exists on the use of the *in vivo* hepatocyte DNA repair assay (1-3, 5, 9, 25-33).

3. Procedure

3.1 Treatment:

3.1.1 All personnel must be knowledgeable in the procedures for safe handling and proper disposal of carcinogens, potential carcinogens, and radiochemicals. Disposable gloves and lab coats must be worn.

3.1.2 Any strain or sex of rat may be used. The largest database is for male Fischer-344 rats. Young adult animals are preferred. It is possible that factors such as sex, age, and strain of the rat could affect the outcome of the DNA repair

¹ This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

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² The boldface numbers in parentheses refer to the list of references found at the end of this practice.