ID 11.3

Assessing DNA damage in Testicular Germ Cells in the Comet Assay

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The in vivo comet assay is a widely used genotoxicity assay, however currently the OECD test guideline 489 (TG489) does not recommend obtaining testicular germ cell data since testicular cell suspensions contain a mixture of germ cells and somatic cells. In absence of the scarcely available in vivo germ cell mutagenicity data, demonstration of interaction with the germ cell genome is warranted for classification as Muta 1B instead of Muta 2 when combined with positive findings of somatic mutagenicity in vivo. An approach to specifically assess testicular germ cells within TG 489 is thus highly demanded. We here provide a proof-of-concept to selectively analyse round spermatids and primary spermatocytes. We utilize semi-automated comet assay recordings of both DNA damage (% tail intensity) and DNA content (total fluorescence intensity) of individual comets, combined with visual comet identification, to distinguish testicular comet populations based on their different DNA content/ploidy and physical appearance. Comet populations are identified through 1) visual discrimination of DNA content distributions, 2) setting DNA content thresholds, and 3) fitting a normal three mixture distribution function. Primary spermatocyte comets can be identified during scoring based on their particularly large physical size combined with high DNA content. We harvested biological materials from an extensive rat experiment testing five classical agents to facilitate inter-laboratory validation. Preliminary data will be presented. Valuable information regarding genotoxic potential as well as distribution of substances to gonads can be gathered. Both somatic and germ cell data can be obtained from the same animals in accordance with the 3R-principle. Our adaptation adds a versatile, sensitive, rapid and resource-effective assay to the currently limited toolbox for regulatory germ cell mutagenicity assessment. Considering the increasing global production of and exposure to potentially hazardous chemicals, new and easily implementable methods are urgently needed. The framework proposed herein may facilitate improved assessment of male germ cell mutagenicity.

Keywords:
Germ cell; OECD TG 489; Mutagenicity; Round spermatid; primary spermatocyte.