Role of pathology peer review in interpretation of the comet assay

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Abstract: When a comet assay, an increasingly popular in vivo genotoxicity test, shows a positive test result, interpretation of that response requires ruling out any confounding tissue site toxicity. Since the comet assay typically uses only two or three daily doses of test agent, precursor tissue changes indicative of toxicity may be easily overlooked. Using case examples for two flavoring agents, perillaldehyde and 4,5-epoxydec-2(trans)-enal, we highlight the role of pathology peer review in verifying precursor tissue changes indicative of tissue site toxicity, thereby increasing confidence in final interpretation of comet assay results. Given global deliberation regarding safety assessment of compounds entering the marketplace, we recommend consideration of pathology peer review for equivocal and positive comet assays so that interpretations are universally consistent. (DOI: 10.1293/tox.2018-0019; J Toxicol Pathol 2018; 31: 155–161)

Key words: 4,5-epoxydec-2(trans)-enal, perillaldehyde, comet assay, pathology peer review, global harmonization

Introduction

Safety assessment of flavoring agents includes successful completion of a battery of in vitro and in vivo genotoxicity assays. When in vitro assays such as the bacterial reverse mutation assay, the in vitro micronucleus assay, or the hypoxanthine phosphoribosyltransferase (HPRT) mutation assay in L5178Y lymphoma cells show positive or equivocal results, regulatory authorities may request an in vivo genotoxicity assay such as a rodent micronucleus and/or comet assay. The comet assay, also known as the single cell gel electrophoresis assay, is a sensitive technique for detection of DNA strand breaks in eukaryotic cells. It may be conducted in mice or rats on most any tissue type and combined with a micronucleus assay and/or integrated into a repeated dose toxicity study. The liver is typically the preferred tissue for evaluation in the comet assay because it is the most active target organ for carcinogenicity; other tissues are selected for evaluation based on any knowledge of a potential mechanism or as site-of-contact tissues most relevant to exposure. As discussed below, histopathology on tissues evaluated in the comet assay represents an important component in interpretation of test results.

Two flavoring agents have recently been tested in genotoxicity assays, including comet assays that heavily relied on histopathology to provide contextual relevance for interpretation of the results. Perillaldehyde (also known as p-mentha-1,8-dien-7-al) is a natural compound in the herb perilla and in the peel of citrus fruits. The isolated chemical from perilla, as well as a chemically synthesized version, is used as a flavoring agent to add a fatty-spicy, oily-herbaceous aroma to a variety of foods and beverages. The second flavoring agent, 4,5-epoxydec-2(trans)-enal, is also a naturally occurring flavoring substance found in various foods/beverages such as orange juice, tea, and dairy products. It can form as a degradation product of fats in many foodstuffs. Because it exists only at a very low level in natural sources, synthesized chemical is used as a flavoring in foods and beverages.

Using perillaldehyde and 4,5-epoxydec-2(trans)-enal as case studies, the purpose of this report is to show the relevance and importance of pathology peer review and to emphasize the need for global harmonization in using pathology peer review as an aid in the evaluation and interpretation of positive comet assay results.
Comet Assay Background

The comet assay is a technique to detect DNA strand breaks in eukaryotic cells. It has gained popularity in genotoxicity testing, especially following acceptance of an Organization for Economic Co-operation and Development (OECD) test guideline for the comet assay in rodents. It is considered a useful follow up test in situations where in vitro genotoxicity assays show positive or equivocal results. It is also often used to meet the second in vivo assay requirement of Option 2 of the standard test battery outlined in the International Conference on Harmonisation (ICH) S2(R1) guidance for genotoxicity testing of pharmaceuticals (http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S2_R1/Step4/S2R1_Step4). Tissue cells to be investigated are embedded in agarose on a microscope slide and their cell membranes lysed with detergent and high salt concentration to form nucleoids containing supercoiled loops of DNA attached to the nuclear matrix. During electrophoresis at high pH, undamaged DNA remains in the nucleoid while negatively charged fragmented DNA migrates out of the nuclear matrix and moves toward the anode creating a trail that, when examined by fluorescence, resembles a comet tail. The intensity and length of the comet tail relative to the comet head that has undamaged supercoiled DNA is an indication of the extent of DNA strand breaks and is assessed microscopically either manually or using image analysis software.

In applying the comet assay as part of a genotoxicity battery of tests, rats or mice are dosed with a test agent of interest and cells are collected for the comet assay shortly after a second or third daily dose. The dosing and harvest regimen is designed to provide sufficient time for metabolism of the test substance while also allowing for detection of strand breaks prior to DNA repair. Multiple dose levels of the test agent plus vehicle and positive (e.g., ethyl methanesulfonate) controls constitute a typical test that often includes clinical chemistry and histopathology to check for any toxicity that could confound results. The comet assay may be combined with an in vivo erythrocyte micronucleus test and/or incorporated into a repeated dose toxicity test to conserve animal use. Details regarding comet assay testing parameters are readily available and will not be covered here.

Interpretation of comet assay results is conventionally based on four parameters:

i. whether or not at least one test dose is statistically significant versus the concurrent control.
ii. whether or not there is a statistically significant positive trend analysis reflecting a dose response.
iii. where data points fall with respect to laboratory historical control data.
iv. demonstration of exposure of the examined tissue(s) to the test agent.

Assessment of exposure/toxicity may be based on clinical signs (e.g., ataxia, piloerection, abnormal or prone posture, loss of body weight, reduced activity), toxicokinetics, clinical chemistry, urinalysis, and histopathology. DNA damage due to chemical-related cytotoxicity or to excessive mechanical disruption during cell isolation can confound interpretation of comet assay results. Therefore, for a given dose to be considered positive, there should be no evidence of excessive toxicity to the examined tissue at that dose. Although the OECD test guideline mandates that the frequency of “hedgehog” cells [a morphology indicative of highly damaged cells which can be associated with severe cytotoxicity, necrosis or apoptosis]; also known as “clouds” and “ghosts”) be determined, there is no consensus as to just how these data should be factored into interpretation of results. The international validation of the in vivo comet assay for the detection of genotoxic carcinogens, coordinated by the Japanese Center for the Validation of Alternative Methods (JaCVAM), led to the conclusion that histopathology remains the “gold standard” for assessing tissue cytotoxicity, and that changes in % tail DNA require careful interpretation when measured in conjunction with severe histopathological changes.

Pathology Peer Review Background

Since pathology diagnosis is qualitative and subjective, a pathology peer review increases confidence in the pathology assessment by assuring the quality of the study animal observations and the pathology diagnoses and their interpretation. The pathology peer review process involves examination of the study pathologist’s (SP) diagnoses and his/her interpretation of pathology study findings by an expert in diagnostic histopathology. The pathology peer review may take place on site at the performing laboratory or remotely and the timing of the review may occur before or after finalization of the SP’s diagnoses and interpretation. In either case the pathology peer review should be planned, managed, and documented.

Although not mandated by Good Laboratory Practice (GLP) guidelines, pathology peer review is endorsed by regulatory authorities as a means for verifying and improving the quality of the pathology data and increasing confidence in the accuracy of the qualitative and subjective nature of pathology diagnoses and interpretation. Recent publications cover procedural considerations and details involved in pathology peer review. There are two situations where regulatory authorities require a pathology peer review. The U.S. Environmental Protection Agency (EPA) requires pathology peer review if a compound is being re-registered and the European Medicines Agency (EMA) requires a pathology peer review for carcinogenicity studies of medicinal products.

Case Examples

Case examples highlighting the importance of pathology peer review are provided for two flavoring agents: perrillaldehyde and 4,5-epoxyde-2(trans)-enal. The European Food Safety Authority (EFSA) evaluation of these two substances was conducted without the benefit of a pathology
Perillaldehyde

Perillaldehyde is a natural organic compound present in the essential oils of fruits, berries and herbs. It is used in perfumery and has a mint-like cinnamon odor. As a flavoring agent it adds a fruity and spicy flavor to a variety of beverages, baked goods, frozen dairy products, gelatins and puddings, and in soft candy.

Perillaldehyde has recently been tested in genotoxicity assays including the comet assay. It is ‘generally recognized as safe’ (GRAS) by the Expert Panel of the U.S. Flavor and Extract Manufacturers Association (FEMA). It is also judged safe by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) and unlikely to harm human health according to the Japanese Food and Sanitation Act (http://www.mhlw.go.jp/english/topics/food-safety/foodd additives/index.html). Perillaldehyde genotoxicity testing results were recently reviewed by a FEMA expert panel that concluded that perillaldehyde has no apparent in vivo genotoxic potential.

Perillaldehyde, a monocyclic terpenoid with an α,β-unsaturated functional aldehyde group, is rapidly metabolized with excretion unchanged or as conjugated carboxylic acid. Based on structural alerts for possible genotoxicity of α,β-unsaturated aldehydes and ketones, perillaldehyde was selected for additional genotoxicity testing as representative of subgroup 2.2 of EFSA’s Flavouring Group Evaluation (FGE.19). The comet assay was among the studies carried out by interested industries. Study details for the comet assay as well as a micronucleus assay in rats, an in vitro HPRT mutation assay in L5178Y mouse lymphoma cells have been published.

Perillaldehyde induced mutations in Salmonella typhimurium strain TA98 without metabolic activation. There was no evidence of genotoxicity in the in vitro micronucleus assay, the HPRT assay, and the micronucleus assay in male Wistar rats.

In combination with the in vivo micronucleus assay, a comet study was conducted in male Wistar rats in accordance with recommendations from FEMA and expert working groups, and generally consistent with the subsequently published OECD guideline 489. Following a dose range-finding study with no gender differences, male rats were administered perillaldehyde by oral gavage at doses of 175, 350 and 700 [the estimated maximum tolerated dose (MTD)] mg/kg/day for three successive days with a corn oil vehicle control and an aqueous ethyl methanesulfonate (150 mg/kg/day) positive control. Animals (6 rats per treatment group) were sacrificed 3 h following the final dose. Blood was collected for clinical chemistry and liver and duodenum tissues were prepared for the comet assay and fixed for histopathology. Comet data were captured using an automated imaging system (Comet Assay IV; Perceptive Instruments, Suffolk, UK).

Results of the comet assay are presented in Table 1.

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>% Tail DNA (mean ± SEM)</th>
<th>% Hedgehogs (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.54 ± 0.15</td>
<td>7.17</td>
</tr>
<tr>
<td>175</td>
<td>0.72 ± 0.11</td>
<td>8.54</td>
</tr>
<tr>
<td>350</td>
<td>0.86 ± 0.11</td>
<td>6.96</td>
</tr>
<tr>
<td>700</td>
<td>2.20 ± 0.60           ***</td>
<td>9.04</td>
</tr>
<tr>
<td>EMS (150)</td>
<td>33.96 ± 1.38           ***</td>
<td>22.48</td>
</tr>
</tbody>
</table>

SEM = standard error of the mean. EMS = ethyl methanesulfonate. ***p<0.001, *p<0.001. Dose response test.

There was no indication of DNA damage in the duodenum of perillaldehyde-treated rats. Liver tissue from rats exposed to the highest dose (700 mg/kg/day) was associated with a small, but statistically significant, increase in % comet tail intensity, and there was a statistically significant linear trend. While the % tail intensities for all 6 rats in the 700 mg/kg/day group were within the laboratory’s historical vehicle control 95th percentile range, the % tail intensity for 5 of these rats exceeded intensity levels in the concurrent controls. A statistically significant increase in DNA damage was measured in the duodenum and liver for the positive control group.

Post dosing clinical observations on day 3 following administration of 700 mg/kg perillaldehyde included a reduced level of activity in 5 of 6 rats and ataxia and piloerection in 1 of 6 rats. No macroscopic changes associated with exposure to perillaldehyde were present. The initial histopathologic findings in liver tissue evaluated by the SP was limited to hepatocyte vacuolation in rats receiving 700 mg/kg/day. In the subsequent pathology peer review of the liver slides, hepatocyte enlargement was present in all 6 rats administered 700 mg/kg/day. In contrast to control rats (Fig. 1), there was periportal to mid-lobular microvesicular cytoplasmic vacuoles consistent with fat, and the hepatocyte enlargement caused compression of sinusoidal channels (Fig. 2). Centrilobular hepatocyte degeneration characterized by loss of cytoplasmic and nuclear detail was occasionally present in these rats. Individual and small aggregates of hepatocytes with condensed nuclear chromatin and hypereosinophilic shrunken cytoplasm were present to varying degree only in the 700 mg/kg/day dose group (Fig. 3). Three of the 6 rats in this highest dose group had high, but not statistically significant, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, indicative of hepatic toxicity. Statistically significant elevation of serum urea and decreased cholesterol, glucose,
and electrolytes (sodium, chloride, potassium) supportive of
general toxicity were also present in the high dose group.
In light of evidence of hepatotoxicity in the 700 mg/kg/day
perillaldehyde rats, and the % tail intensity results falling
within laboratory historical data, the comet results in this
group are considered secondary to toxicity and not a direct
effect of perillaldehyde.

4,5-Epoxydec-2(trans)-enal

4,5-Epoxydec-2(trans)-enal is used as a fragrance and
flavoring agent in a variety of foods including canned or-
ange juice, breads, various cooking oils, buttermilk, boiled
cod, and popcorn. It is used to enhance the flavor and/or
odor of a variety of foods and has a citrus odor and fruity
mandarin orange flavor. It can be formed during baking
with fats containing linoleic acid.

4,5-Epoxydec-2(trans)-enal was evaluated for geno-
toxicity as a representative of subgroup 1.1.1(b) of FGE 19
in FGE 226 aliphatic aldehydes with α,β-unsaturation con-
jugated with an epoxide moiety that is considered a struc-
tural alert. It was previously considered to have no safety
concerns at current levels of intake by JECFA, 20. As ref-
denced by EFSA, a bacterial reverse mutation assay was
negative and in vitro micronucleus assays in human peripheral
lymphocytes gave equivocal, positive, and negative re-
results in successive assays. An in vivo micronucleus assay
conducted in Han Wistar rats, while negative for genotoxic-
ity, was considered unreliable since there was no evidence
of bone marrow exposure. A rat comet assay was recom-
ended by EFSA.

For the comet assay, an MTD of 350 mg/kg/day was
determined based on a dose range-finding study in Han
Wistar rats with no gender difference in response. Con-
sequently, a comet assay was designed with 6 male rats per
group. Groups included a 0.5% aqueous methylcellulose
vehicle control, an ethyl methanesulfonate positive control
group (150 mg/kg), and treatment groups administered 75,
150 and 300 mg/kg/day of 4,5-epoxydec-2(trans)-enal by
oral gavage. Rats were sacrificed 3 h following the second
day of dosing, blood was collected for clinical chemistry,
and liver and duodenum tissues were prepared for the comet
assay and fixed for histopathology. Comet data were cap-
tured using an automated imaging system (Comet Assay IV;
Perceptive Instruments). Study design details are provided
in a company report.

Comet assay results for the duodenum were negative
data not shown). Results of the comet assay for liver are
presented in Table 2. A statistically increased mean % tail
intensity was observed in groups receiving 75 and 300, but
not 150, mg/kg/day of 4,5-epoxydec-2(trans)-enal with a
statistically positive trend and with all individual values
falling well within the laboratory’s historical control 95%
reference range.

There was a small dose-related reduction in body
weight gain resulting in body weight loss at the 300 mg/
kg/day dose group. There was a dose-related increase in se-
rum albumin, total protein, the albumin/globulin ratio and
serum phosphorus and a dose-related decreased mean se-
rum calcium. One rat given 300 mg/kg/day of 4,5-epoxyde-
-2(trans)-enal had a marked increase in serum urea and
creatinine and the highest serum AST and ALT, with the
ALT 3 times higher than the control group mean ALT. In
addition, small increases in serum urea were recorded in
several rats administered 300 and 150 mg/kg/day of 4,5-ep-
oxidec-2(trans)-enal. Since the rats were not fasted before
termination, control livers contained irregular clear cyto-
plasmic spaces consistent with glycogen. The SP
reported a minimal to slight decreased hepatocyte glycogen
in all 4,5-epoxydec-2(trans)-enal treated groups with a dose
response. During the pathology peer review, liver
changes were noted in the rats administered 300 mg/kg/day
where there was a prominent increase in hypereosinophilic
hepatocytes in multiple areas in both liver sections provided
for each of the 6 rats. The hypereosinophilic hepa-
tocytes had angular cytoplasmic membranes, condensed
hyperchromatic nuclei, shrunken and mis-shaped nuclei,
and often had evidence of condensed ribosomal cytoplas-
mic deposits. These liver changes represent features of
progression from early degeneration to cell death. Clear
evidence of centrilobular hepatocyte necrosis was present
in one of the six high dose rats. It is noted that one
rat in each of the lower doses had rare occurrence of hy-
pereosinophilic hepatocyte cytoplasm, likely representing
biological variability in susceptibility. Taken together, the
clinical signs, clinical chemistry changes and morphologi-
cal evidence of hepatocellular degeneration and necrosis re-
fect hepatotoxicity at the 300 mg/kg/day dose level.

Discussion

Current genotoxicity test guidelines mandate that,
unless precluded by use of the limit dose, exposure of an
examined tissue to the test agent be demonstrated. In lieu
of direct measurement of a test agent or its metabolite in
tissues by bioanalytical methods, which can be costly and
burdensome, this criterion for a valid test may sometimes be
achieved by demonstrating adverse clinical signs indicative
of reaching the MTD and/or detection of evidence of toxic-
ity in relevant tissues. Since toxicity can lead to confound-
ing results in the comet assay, accurate assessment of tissue
cytotoxicity is imperative. During the JaCVAM validation
trial of the in vivo comet assay, a standardized approach for
presenting and interpreting histopathological findings was
deemed necessary. It was concluded that necrosis and/or
findings indicative of degenerative changes would be the
main indicators of cellular toxicity, whereas apoptosis could
indicate both cellular toxicity and DNA damage. Further-
more, it was determined that grade and incidence of histo-
pathological changes should be considered in the interpreta-
tion of cytotoxicity in relation to positive comet results.

The conduct of the comet assay typically involves a
relatively short 2- or 3-day exposure to a test agent with-
out sufficient time for classical hepatotoxicity to be clearly
Fig. 1. Irregular clear spaces in hepatocyte cytoplasm are consistent with intracytoplasmic glycogen storage in this liver from a vehicle control rat.

Fig. 2. Hepatocytes are enlarged with small inform vacuoles consistent with microvesicular fat filling and expanding the cytoplasm and surrounding centrally located nuclei. Liver from a rat sacrificed 3 h after third daily gavage dose of 700 mg/kg of perillaldehyde.

Fig. 3. Hypereosinophilic hepatocytes with condensed to pyknotic nuclei and shrunken angularly shaped cytoplasm (arrows) are randomly distributed among pale staining hepatocytes that are enlarged with cytoplasmic microvesicular fat vacuoles. The shrunken hypereosinophilic hepatocytes reflect different stages of hepatocellular death. Liver is from a rat sacrificed 3 h after third daily gavage dose of 700 mg/kg of perillaldehyde.

Fig. 4. Normal liver with irregular clear areas in cytoplasm consistent with glycogen storage. Liver is from a vehicle control rat.

Fig. 5. A normal area of liver showing absence of cytoplasmic glycogen from a rat sacrificed 3 h after a second daily dose of 300 mg/kg of 4,5-epoxydec-2(trans)-enal.

Fig. 6. Multiple hypereosinophilic hepatocytes with condensed cytoplasm, angular shapes, and condensed to pyknotic nuclei (arrows) from a rat sacrificed 3 h after a second daily dose of 300 mg/kg of 4,5-epoxydec-2(trans)-enal. The shrunken hypereosinophilic hepatocytes reflect different stages of hepatocellular death.

Fig. 7. Higher magnification of Figure 6 showing details of shrunken dying hepatocytes (arrows) from a rat sacrificed 3 h after a second daily dose of 300 mg/kg of 4,5-epoxydec-2(trans)-enal.

Fig. 8. A focal area of centrilobular hepatocellular necrosis with loss of normal hepatocellular structure and cells (asterisk). Liver from a rat sacrificed 3 h after a second daily dose of 300 mg/kg of 4,5-epoxydec-2(trans)-enal.
3,250 flavoring substances are in use to modify flavor and/or aroma. In a 2015 publication, it was estimated that about 3,250 flavoring substances are in use in food and beverages. Many of these foods and beverages contain flavoring substances that impart, enhance and/or modify flavor and/or aroma. In a 2015 publication, it was estimated that about 3,250 flavoring substances are in use in Japan, 2,500 in Europe, and 2,300 in the USA. Genotoxicity assays are a component of the safety assessment of food additives such as flavoring agents and are conducted in various countries. The Konishi et al. publication indicates the need and importance of international harmonization of these as well as other safety assessments. Given global considerations regarding safety assessment of compounds entering the marketplace, it is important to harmonize standards for conduct of pathology peer review such that the process is transparent, and interpretations are consistent.

The two flavoring agents highlighted in this report are each considered representative of a class of flavoring substances. Consequently, the outcome of their hazard assessment has implications for the remaining members of their respective chemical classes. Perillaldehyde and 4,5-epoxydec-2(\(\text{trans}\))-enal were evaluated in Europe by EFSA and judged to have genotoxic potential. At the same time, these two flavoring agents are permitted in food products in the USA and Japan where they are judged to be safe at current usage levels by regulatory authorities and scientific bodies such as JECFA, FEMA Expert Panel, U.S. Food and Drug Administration (FDA) and Japanese Ministry of Health, Labour and Welfare (MHLW). The EFSA judgment that these two flavoring agents have genotoxic potential, based on statistically positive comet assays, and determined without the benefit of a pathology peer review that identified hepatotoxicity, stands in contrast to the opinions of these other scientific bodies. Whether having the findings of the pathology peer review histopathology and interpretation in advance would have influenced the EFSA judgment regarding the genotoxicity hazard of perillaldehyde and 4,5-epoxydec-2(\(\text{trans}\))-enal remains an open question.

Table 2. Comet Assay Results for Male Rats Administered 4,5-epoxydec-2(\(\text{trans}\))-enal

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>% Tail DNA (mean ± SEM)</th>
<th>% Hedgehogs (Mean)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0.24 ± 0.07</td>
<td>1.17</td>
</tr>
<tr>
<td>75</td>
<td>0.83 ± 0.25*</td>
<td>3.48</td>
</tr>
<tr>
<td>150</td>
<td>0.46 ± 0.23</td>
<td>1.93</td>
</tr>
<tr>
<td>300</td>
<td>1.06 ± 0.24**</td>
<td>2.68</td>
</tr>
<tr>
<td>EMS (150)</td>
<td>16.28 ± 1.24***</td>
<td>2.96</td>
</tr>
</tbody>
</table>

SEM = standard error of the mean. EMS = ethyl methane-sulfonate. *p<0.05, **p<0.01, ***p<0.001, #p<0.05. Dose response test.

In the two examples highlighted in this report, EFSA evaluated the results of the comet assays based on the available information at the time of their final determination. EFSA did not have the benefit of the pathology peer review findings of histopathological hepatic changes when evaluating perillaldehyde and 4,5-epoxydec-2(\(\text{trans}\))-enal comet assay data. The results of genotoxicity testing of perillaldehyde, including the pathology peer review findings, were recently reviewed by a FEMA expert panel that concluded there is no apparent in vivo genotoxic potential. As indicated earlier, 4,5-epoxydec-2(\(\text{trans}\))-enal was previously considered to have no safety concerns at current use levels.

Contemporary growth of international commerce has fostered a consumer interest in ethnic food products and allowed for global availability of ethnic and country-specific foods and beverages. Many of these foods and beverages contain flavoring substances that impart, enhance and/or modify flavor and/or aroma. In a 2015 publication, it was estimated that about 3,250 flavoring substances are in use in Japan, 2,500 in Europe, and 2,300 in the USA. Genotoxicity assays are a component of the safety assessment of food additives such as flavoring agents and are conducted in various countries. The Konishi et al. publication indicates the need and importance of international harmonization of these as well as other safety assessments. Given global considerations regarding safety assessment of compounds entering the marketplace, it is important to harmonize standards for conduct of pathology peer review such that the process is transparent, and interpretations are consistent.

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Clearly, the field would benefit from a greater mechanistic understanding of the specific histopathological findings that correlate with confounding effects on the comet assay. Moreover, improved harmonization of testing and data interpretation standards for safety assessment as well as submission of peer-reviewed subjective study results will lead to greater confidence in data and better regulatory decisions in hazard identification. Towards that end, we believe there is an important role for pathology peer review in safety assessment of food additives, including flavorings, as well as other test agents that have a positive or equivocal comet assay response.

Disclosure of Potential Conflicts of Interest: The authors have no thing to declare.

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