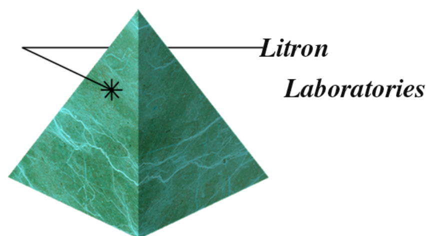

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February 8, 2011

Dear Ms. Kennedy,

I am writing this letter to comment on the draft UK COM paper “Guidance on a Strategy for Genotoxicity Testing and Mutagenic Hazard Assessment of Chemical Substances”. Although my name does not appear on your consultant list, I hope the ability to comment remains open.

I would like to start out by making you aware that my current position is Director of Research, Litron Laboratories, Rochester, N.Y. Litron holds several patents in the area of genetic toxicology methods, and sells flow cytometry-based genotoxicity kits (*in vitro* and *in vivo* micronucleus scoring, trade name MicroFlow®). The company also has plans to develop kit(s) based on gene mutation at the *Pig-a* locus. I am making these disclosures because they could be viewed as biases. Even so, I would like to take this opportunity to share several comments/suggestions with you, hoping they will be carefully considered before you finalize your document.

You will find my comments/suggestions over the next several pages. I would be happy to discuss or clarify these with UK COM as necessary. Thank you for providing this opportunity.

Sincerely,

Stephen D. Dertinger, Ph.D.
Director of Research
Litron Laboratories

1) I note that throughout the document there is language similar to that found on pg. 6, lines 3-5: “The objective of genotoxicity testing is to exclude or identify potential mutagenic hazards to humans, and for those that are positive, to aid in the elucidation of the mode of genotoxic action (MoGA).”

I agree with these sentiments, and will comment on MoGA once again below. Currently, I’d like to inquire whether UK COM has considered adding language that more fully embraces the role that MoGA, in conjunction with well designed studies and quantitative analyses, may have in risk assessment. As the relatively recent Roche/Viracept example shows, the future of genetic toxicology may be evolving from a “screen and bin” approach (genotoxic yes or no) to a discipline that is grounded in the toxicological principle “the dose makes the poison”.

For example, the following is from a recent manuscript by Pottenger and Gollapudi (Environ. Mol. Mutagen. 51 (2010) 792-799):

> “We have stated at the outset that NOGEL and threshold values derived from multiple test systems, when examined collectively, would inform the risk assessor on the likelihood of an adverse effect at a given human exposure level. This approach, illustrated in Figure 2, will take genetic toxicology testing beyond the hazard-based ‘screen and bin’ approach and will move the field more decisively towards informing the risk assessment process.”

It appears to me as though this evolving use of genetic toxicology assays/endpoints is not fully captured by your draft document, and I wonder to what extent UK COM has contemplated a more thorough discussion on this topic.

2) I note that throughout the document there is language similar to that found on pg. 6, lines 7-11: “Consequently, it is important to generate information on three levels of genetic damage, namely gene mutation, chromosome structure (i.e. clastogenicity) and chromosome number (i.e. aneuploidy), to provide comprehensive coverage of the mutagenic potential of a chemical.”

Given the document’s emphasis on comprehensive coverage of genetic damage, as well as its specific support of *in vivo* transgenic rodent models, it is somewhat surprising to me that the *in vivo Pig-a* assay does not make an appearance until pg 44. Perhaps the use of two somewhat older citations helps explains this. In any event, I would like to take this opportunity to update UK COM on the fact that an international, interlaboratory (20 sites) ring trial is underway to test the portability of the *Pig-a* assay and to investigate more chemicals in this system. Likewise, the amount of work being done with this assay also becomes apparent when one considers a more updated list of papers in this subject area:

S. Phonethepswath, S.M. Bryce, J.C. Bemis, S.D. Dertinger (2008) Erythrocyte-based *Pig-a* gene mutation assay: Demonstration of cross-species potential, Mutat. Res. 657, 122–126.

D. Miura, V.N. Dobrovolsky, T. Kimoto,Y. Kasahara, R.H. Heflich (2009) Accumulation and persistence of *pig-a* mutant peripheral blood cells following treatment of rats with single and split doses of *N*-ethyl-*N*-nitrosourea, Mutat. Res. 677, 86–92.
V.N. Dobrovolsky, J.G.Shaddock, R.A. Mittelstaedt, M.G. Manjanatha, D. Miura, M. Uchikawa, D.R. Mattison, S.M. Morris (2009) Evaluationof *Macac mulatta* as a model for genotoxicity studies, Mutat. Res. 673, 21–28.
S. Phonethepswath, D. Franklin, D.K. Torous, S.M. Bryce, J.C. Bemis, S. Raja, S. Avlasevich, P. Weller, O. Hyrien, J. Palis, J.T. MacGregor, S.D. Dertinger (2010) *Pig-a* mutation: Kinetics in rat erythrocytes following exposure to five prototypical mutagens, Tox.Sci. 114, 59–70.
S.D. Dertinger, S. Phonethepswath, D. Franklin, P. Weller, D.K. Torous, S.M. Bryce, S. Avlasevich, J.C. Bemis, O.Hyrien, J. Palis, J.T. MacGregor (2010) Integration of mutation and chromosomal damage endpoints into 28-day repeat dose toxicology studies, Tox. Sci.115, 401–411.
V.N. Dobrovolsky, D. Miura, R.H. Heflich, S.D.Dertinger (2010) The *in vivo pig-a* gene mutation assay, a potential tool for regulatory safety assessment, Environ. Mol. Mutagen. 51, 825–835.
S.D. Dertinger, S.M. Bryce, S. Phonethepswath, S. Avlasevich (2011) When pigs fly: Immunomagnetic separation facilitates rapid determination of *Pig-a* mutant frequency by flow cytometric analysis, in press, Mutat. Res. DOI: 10.1016/j.mrgentox.2011.01.009.

I am aware of at least 12 other papers that are in preparation, as Dr. Robert Heflich (FDA-NCTR) and I are serving as guest editors of a Special Issue of Environ. Mol. Mutagen. that will be dedicated to the *in vivo Pig-a* gene mutation assay.

These reports continue to support the sentiment of Dertinger *et al.* (Mutat. Res., DOI: 10.1016/j.mrgentox.2011.01.009):

> "Over the last several years the genetic toxicology community has expressed considerable enthusiasm for the *in vivo Pig-a* mutation endpoint [11–14]. Some of the most compelling factors that are driving interest in these assays are the following: (i) there is no need to grow cells *ex vivo* to measure the frequency of mutant phenotype cells, (ii) while mutation is a key event in carcinogenesis, most *in vivo* mutation assays have cost/technical limitations, (iii) *Pig-a*-based methods provide a means of studying mode of action and dose-response relationships which are important for risk assessment, (iv) unlike clastogenicity endpoints, *Pig-a* mutant cells accumulate with repeated dosing [7], (v) cross-species potential is high [6,8,12], and (vi) the endpoint integrates well with repeat-dosing experimental designs, for instance those commonly used in general toxicology studies 374 [10]."

3) Whereas IWGT and draft ICH guidance documents, as well as other reports, explicitly support the use of the rat peripheral blood compartment for measuring micronucleated reticulocytes (MN-RET), it appears as though the UK COM document is less clear. For instance, there is language on page 38 that states peripheral blood is an "**alternate** approach for both mice and rats", and the heading of this same section is

"*Rodent **Bone Marrow** MN and…"*. In my opinion this does not give the reader confidence in the suitability of peripheral blood the way more careful phrasing would afford. For instance, I suggest avoiding the use of the term "alternate". Also, the header could refer to "hematopoietic cells" as opposed to "bone marrow". These changes would serve to diminish the historical bias that exists against the blood compartment as opposed to reinforcing it as the current language does.

Similarly, it is not clear to me how well the current phrasing supports the use of blood MN-RET endpoint derived from general toxicology studies. This is potentially very important, as it speaks to the UK COM's desire to limit and refine the use of animals in testing. For instance, in my opinion the language used by Pfuhler and colleagues (Regulatory Toxicology and Pharmacology, 57 (2010) 315-324) is clearer on this matter:

> "The integration of the micronucleus (MN) endpoint into RDT testing is a long and well established concept (Wakata *et al.,* 1998; Hayashi *et al.,* 2000, 2007; Hamada *et al.,* 2001) and is already represented in the current OECD guideline, which came into effect in 1997 (OECD TG 474). The fact that micronuclei can be scored in peripheral blood using flow cytometry does simplify this approach, and the above mentioned studies did show that peripheral blood is equally as reliable as the bone marrow for measuring micronuclei even in rats that exhibit an efficient splenic filtration function. The integration of the MNT is also recognised by the draft ICH S2 (R1) guideline, which states that 'the *in vivo* genotoxicity assays can often be integrated into RDT studies when the doses are sufficient' (ICH, 2008). At an ECVAM workshop in June 2008, the possibility to integrate the MNT and Comet assays into RDT studies was thoroughly discussed and the MNT was considered as scientifically credible and therefore ready for use in RDT studies (Pfuhler *et al.,* 2009)."

4) Whereas the draft UK COM document discusses the fact that flow cytometry has been successfully applied to scoring micronuclei in cell cultures (pgs. 26-27), it leaves off with the sentiment "…further validation of the FCMMN assay is required before it can be used for regulatory submissions." I would like to use this opportunity to update UK COM group on several very recent reports that advance validation efforts:

J. Shi, R. Bezabhie, A. Szkudlinska (2010) Further evaluation of a flow cytometric in vitro micronucleus assay in CHO-K1 cells: a reliable platform that detects micronuclei and discriminates apoptotic bodies, *Mutagenesis,* 25, 33-40.

S.M. Bryce, S.L. Avlasevich, J.C. Bemis, S. Phonethepswath, S.D. Dertinger (2010) Miniaturized flow cytometric in vitro micronucleus assay represents an efficient tool for comprehensively characterizing genotoxicity dose-response relationships, *Mutat. Res.,* 703, 191-199.

M. Lukamowicz, K. Woodward, M. Kirsch-Volders, W. Suter, A. Elhajouji (2011) A flow cytometry based in vitro micronucleus assay in TK6 cells—Validation using early stage pharmaceutical development compounds, *Environ. Mol. Mutagen.,* in press, DOI: 10.1002/em.20632.

J. Nicolette, M. Diehl, P. Sonders, S. Bryce, E. Blomme (2011) In vitro micronucleus screening of pharmaceutical candidates by flow cytometry in Chinese hamster V79 cells, *Environ. Mol. Mutagen.,* in press, DOI: 10.1002/em.20631.

S.M. Bryce, S.L. Avlasevich, J.C. Bemis, S.D. Dertinger (2011) Miniaturized flow cytometry-based CHO-K1 micronucleus assay discriminates aneugenic and clastogenic modes of action, *Environ. Mol. Mutagen.,* in press, DOI: 10.1002/em.20618.

Thank you again for providing this opportunity to comment on your document.

Respectfully submitted, Stephen D. Dertinger