Message

From: [redacted]@bham.ac.uk]
Sent: 6/24/2009 8:22:30 AM
To: SALTMIRAS, DAVID A [AG/1000] [redacted]@monsanto.com]
Subject: Re: Latest Seralini

Hi David
Had a chance to read this, basically more of the same i.e. over inflation of relevance of claims derived from a poor in vitro model to the in vivo situation. See below for specific comments. In the discussion they make the usual invalid claims about linking levels applied to crops and authorised food levels to their in vitro studies implying that these are likely concentrations that could be achieved in cells in vivo, this is clearly not true in my opinion. They also relate their observations back to levels of G when it is clear that it is not G that is responsible for the effects so this comment is not relevant.

1) The cytotox data as in all these studies clearly show that it is the adjuvants not G that is responsible for the cytotoxicity and presumably the other effects although in this paper they only test R400 and not G alone for other endpoints (caspase 3/7, comet assay, endocrine disruption assays). To me as already discussed this points to an non specific [toxic] effect related to the detergent properties of the additives.

2) I still have methodological doubts about their model especially the use of serum free media [24h] for treatments, this is non standard, probably stresses the cells regardless of any treatment and is a completely non physiological situation because it is likely that these agents are likely to bind to serum proteins etc in the media (and in plasma in vivo) restrict access to cells. There are also the concerns remaining about media pH.

3) I was very surprised to see the comet data which is suggestive of genotoxicity. However I do have some serious concerns about this data:

The way the data is presented is not standard, Data is normally presented as median tail % + SD (see Duez et al, 2003,Mutagenesis 2:p159-66) as it is presented in paper there is no indication of the variation within the data nor is there any statistical analysis to substantiate their claims. In addition, some of the buffers used in the comet assay are also poorly defined e..g the electrophoresis buffer which is just stated as pH13 buffer (this is a normal pH for this buffer but clearly it would be useful to know what it is!!), the unwinding time used was 40 min rather than the more standard 20min and they don't quote the electrophoresis conditions in terms of volts/cm which is typical to do.

Having said all of that there does seem to be a clear concentration effect. I was very surprised to see a similar response at the top dose of R400 to the Benzopyrene (50uM) positive control. When i looked into this further it seems that the dose of BP chosen (50uM) is likely to directly toxic to HepG2 cells. In fact other groups have found that BP >10uM is toxic to Hep G2 cell

e.g. Park SY, Lee SM, Ye SK, Yoon SH, Chung MH, Choi J. Toxicol Lett. 2006 Nov 1;167(1):27-33. Epub 2006 Sep 3. Benzo[a]pyrene-induced DNA damage and p53 modulation in human hepatoma HepG2 cells for the identification of potential biomarkers for PAH monitoring and risk assessment.

Seralini fails to report whether BP (50uM) is toxic to cells in his study at this dose but i strongly suspect it is. This would invalidate its use as a positive control because damage would likely just be a result of DNA fragmentation during apoptosis/necrosis after treatment with a toxic dose of BP.

This still fails to explain the positivity seen with R400 in the absence of apparent toxicity, we could include some comet assay studies in the proposed work we are going to do as this is something we do routinely at Birmingham. They also imply in their discussion that oxidative stress may be the mechanism

we could address this directly by use of the modified fpg-comet assay if
needed.

[redacted]

On Tuesday 23 June 2009 21:01:53 you wrote:
>Hi [redacted]
>
>Do you have any comments on the Seralini paper? Any feedback would be
> helpful. We are issuing a response/position to our Brussels office on
> Friday.
>
>Cheers,
>
>David Saltmiras, Ph.D., D.A.B.T.
>Toxicology Manager
>Regulatory Product Safety Center
>Monsanto
>ph [redacted]
>
>
>-----Original Message-----
>From: [redacted] [mailto:[redacted]@bham.ac.uk]
>Sent: Friday, June 19, 2009 10:29 AM
>To: SALTMIRAS, DAVID A [AG/1000]
>Subject: RE: Latest Seralini
>
>HI David
>will try and look at the paper over the weekend
>
>best wishes
>[redacted]
>
>On Fri, 2009-06-19 at 09:46 -0500, SALTMIRAS, DAVID A [AG/1000] wrote:
>> [redacted]
>>
>> In light of the new Seralini paper, so you see any relevant refinements to
>> your research proposal?
>>
>> Also, a European colleague just told me that in the UK, Friday is POETS
>> day (piss off early tomorrow's Saturday).
>>
>> Cheers,
>>
>> David Saltmiras, Ph.D., D.A.B.T.
>> Toxicology Manager
>> Regulatory Product Safety Center
>> Monsanto
>> ph [redacted]
>>
>>
>> -----Original Message-----
>> From: [redacted] [mailto:[redacted]@bham.ac.uk]
>> Sent: Friday, June 19, 2009 3:06 AM
>> To: SALTMIRAS, DAVID A [AG/1000]
>> Subject: Re: Latest Seralini
>>
>> Hi David
>> thanks for this, yes it will be good to make a start on this work
>>
>> best wishes
>>
>> [redacted]
>>
>> On Thu, 2009-06-18 at 13:12 -0500, SALTMIRAS, DAVID A [AG/1000] wrote:
>> > [redacted] & [redacted],
>> >
>> > FYI – the latest Seralini publication referenced below.

>> >
>> > Céline Gasnier, Coralie Dumont, Nora Benachour, Emilie Clair,
>> > Marie-Christine Chagnon and Gilles-Eric Séralini. (2009)
>> >
>> > Glyphosate-based herbicides are toxic and endocrine disruptors
>> > in human cell lines.
>> >
>> > Toxicology, In Press, Accepted Manuscript, Available online 17
>> > June 2009. doi:10.1016/j.tox.2009.06.006
>> >
>> >
>> > Also, I believe our contract is in final approval stages, allowing you
>> > to soon provide an initial invoice to procure supplies and start your
>> > investigations.
>> >
>> >
>> > Regards,
>> >
>> > David
>> >
>> > David Saltmiras, Ph.D., D.A.B.T.
>> > Toxicology Manager
>> > Regulatory Product Safety Center
>> > Monsanto
>> > ph [redacted]
>> >
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