Message

From: FARMER, DONNA R [FND/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=180070]
Sent: 5/12/2000 2:23:27 PM
To: BUNCH, RODERICK T. [PHR/1825] [/O=MONSANTO/OU=NA-1825-01/cn=Recipients/cn=112309]
CC: KIER, LARRY D [NCP/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=33322]; HEYDENS, WILLIAM F [AG/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=230737]; MARTENS, MARK A [FND/5045] [/O=MONSANTO/OU=EA-5040-01/cn=Recipients/cn=21606]
Subject: RE: A couple of things!

Todd,

Have you been able to schedule the retest of the two surfactants in a more definitive type of study?

Thanks,

Donna

-----Original Message-----
From: FARMER, DONNA R [FND/1000]
Sent: Thursday, April 27, 2000 2:37 PM
To: BUNCH, RODERICK T. [PHR/1825]
Cc: KIER, LARRY D [NCP/1000]; HEYDENS, WILLIAM F [AG/1000]; MARTENS, MARK A [FND/5045]
Subject: RE: A couple of things!

Todd,

Thanks for the followup. I forwarded your message to Larry, Bill and Mark.

We would like to retest the tallowamine and the C12 alkylsulfate. It appears a simple repeat of the study using the rapid screen protocol will not help us understand what is going on here, we need a more definitive type of study. Would it be possibly to design and conduct the experiment tailored to the existing data . Maybe changes in replications and/or dose level selections etc.

Any suggestions/thoughts?

Thanks,

Donna

-----Original Message-----
From: BUNCH, RODERICK T. [PHR/1825]
Sent: Tuesday, April 18, 2000 10:57 AM
To: FARMER, DONNA R [FND/1000]
Subject: RE: A couple of things!

Donna,
Attached is an excel data file that Cindy prepared.

You will notice that while scoring for clastogenic/aneugenic potential by looking for binucleated cells with micronuclei (Mn-Bi's), we also score uninucleated cells (uni's), binucleated cells (Bi's), and multinucleated cells (>2's). IF cells divide during the treatment period, they will be Bi's, if they divide more than once they will be >2's. If they don't divide they will be uni's. The cytochalasin B inhibits real cell division, so I really mean nuclear division. If the drug inhibits growth or is cytotoxic, you will see an increase in uni's and/or a decrease in >2's. The Bi's is always 200 as that is the goal for scoring.

Please note that the control range for Mn-Bi's is 0-4 per 200 Bi's for this data set. Our threshold for positive response is 8. The effects of the C12 and the tallow compound seem test article related, but clearly not a robust clastogenic response.

I talked to a couple other companies recently an P&G said they have, on rare occasion, had micronuclei positive

surfactants. We can talk more about this later.

As an aside, we are developing a kinetochore staining assay to differentiate between clastogens and aneugens, either of which could induce micronuclei. This may not be helpful when only an equivocal response is observed, but we could discuss this further if you wish.

Let me know if you have any questions.
Todd

<< File: surfdatasum.xls >>

-----Original Message-----
From: FARMER, DONNA R [FND/1000]
Sent: Friday, April 14, 2000 7:07 AM
To: BUNCH, RODERICK T. [PHR/1825]
Cc: HEYDENS, WILLIAM F [AG/1000]
Subject: FW: A couple of things!

Todd,

Regarding the uMN results are there data tables we can take a look at? Any other thoughts on this since we last spoke?

Thanks,

Donna

-----Original Message-----
From: HEYDENS, WILLIAM F [AG/1000]
Sent: Thursday, April 13, 2000 5:57 PM
To: FARMER, DONNA R [FND/1000]; KIER, LARRY D [NCP/1000]; MARTENS, MARK A [FND/5045]
Subject: RE: A couple of things!

Donna,

1) I do have copies of the clin chem. tables for the "pre-UDS" liver tox. study. I remember you showing the numbers to me and not being real impressed by them. I don't feel strongly about doing histo given the data.

2) Do you have data tables for the micro-MN results ? It would be nice to see what kind of numbers/results we are really looking at here.

Bill

-----Original Message-----
From: FARMER, DONNA R [FND/1000]
Sent: Thursday, April 13, 2000 10:19 AM
To: KIER, LARRY D [NCP/1000]; MARTENS, MARK A [FND/5045]; HEYDENS, WILLIAM F [AG/1000]
Subject: A couple of things!

1) Have all of you had a chance to look at the clin chem tables from SRI on the toxicity studies with glyphosate and MON 52276.

I asked them to give us their interpretation of the results and this is what they said:

Donna,

I would interpret the data to indicate a modest effect on the liver at 3 and 12
hours, which could be due to an increase in protein synthesis. While there are
statistically significant changes all the parameters do fall within normal
historical ranges so the changes are probably of limited biological relevance
and not an indication of a toxic effect.

Chris

When discussing whether we do histopath or not - Chris said he talked to the pathologist and their opinion is- since these fell within normal historical ranges and were not statistically significant by 48 hrs - we would not see anything.

Question???? Do we want to do histopath? If so do we do all dose leves? or just control and high-dose?

2) Below I have included a number of messages on the micromicronucleus studies. What is your opinion of the results? Where do we stand with sending these to Dr. Parry.

Thanks,

Donna

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See messages below - according to Todd Bunch there was a decrease in cells with multiple nuclei for the tallowamine and alkyl sulfate assays. It was below their threshold for a positive response but was biostatistically different from concurrent and historical controls, that is why they called it equivocal but test-related. It appears that the changes induced in this study are changes in cell cycle kinetics and that those can be changed by toxicity as well and we certainly have toxicity in these assays.

-----Original Message-----
From: FARMER, DONNA R [FND/1000]
Sent: Thursday, March 16, 2000 9:23 AM
To: BUNCH, RODERICK T. [PHR/1825]
Subject: RE: uMN results for surfactants

Todd,

As a followup-up to my voice message. My concern is that in the first assay the results for the tallowamine and alkyl sulfate appeared to be negative, in the second they are considered equivocal but test-related and with the cocoamine we did not re-run it and it stayed a negative.

We have negative ames and negative mouse in vivo micronucleus with a tallowamine surfactant. Are these truely mutagenic responses or because of the cytotoxicity properties are we looking at secondary effects such DNA damage thru cell death.

I would appreciate your review of the data and opinion of what we are looking at here.

Thanks,

Donna

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Todd,

As I have not heard from you regarding these assays I will try to call you tomorrow.

Donna

-----Original Message-----
From: FARMER, DONNA R [FND/1000]
Sent: Thursday, March 09, 2000 3:05 PM
To: BUNCH, RODERICK T. [PHR/1825]
Cc: MCADAMS, JAMES G [PHR/1000]; GROSS, CINDY JO [PHR/1000]; ASBURY, KAREN J [PHR/1735]
Subject: RE: uMN results for surfactants

-----Original Message-----
From: GROSS, CINDY JO [PHR/1000]
Sent: Wednesday, March 08, 2000 4:03 PM
To: FARMER, DONNA R [FND/1000]
Cc: BUNCH, RODERICK T. [PHR/1825]; MCADAMS, JAMES G [PHR/1000]
Subject: uMN results for surfactants

Todd,

Below I have cut and pasted 3 summaries regarding surfactants we requested be run in the in situ micronucleus assay. The first paragraph is a paragraph from Karen regarding a cocoamine surfactant, the second is from the first run of 4 other surfactants and the last is the repeat assays with those 4 surfactants.

I would like to discuss these in detail with you (or who ever is the appropriate person (s)). In addition if possible I would like to see the "raw" data tables in order to better understand concentration ranges and the results.

Please give me a call and let's talk about how we proceed.

Thanks,

Donna [redacted]

(1) In Situ Micronucleus
Cocoamine surfactant was tested in the in situ micronucleus assay from 7-21-99 at concentrations ranging from 7.81-500 mg/ml (24 mM - 1.5 mM) without and with metabolic activation. Countable concentrations were scored for three dosage levels without and with metabolic activation. Countable concentrations were at all levels. No evidence of micronucleus induction was observed with the compound under both metabolic activation scenarios. The negative and positive controls without and with metabolic activation demonstrated that this system was capable of detecting direct-acting and metabolism-dependent chemical clastogens in all experiments. Therefore, this compound did not induce micronuclei in CHO-WBL cells under the conditions of this assay system.

(2) Jim ran the benzalkonium chloride, tallow amine, C12 alkyl sulfate, and MON59117 in the in situ micronucleus assay on 1/10 at concentrations of 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, and 500 ug/ml with metabolic activation. Countable concentrations were scored for five dosage levels for benzalkonium chloride (500, 125, 15.6, 7.8, and 3.9 ug/ml); no evidence of micronucleus induction was observed with the compound at these doses. Unfortunately, with the tallow amine, cytotoxicity was observed for all doses tested except 7.8 and 3.9 ug/ml; similarly, with the C12 alkyl sulfate and MON59117 cytotoxicity was observed for the top four doses (500 through 62.5 ug/ml). While the lower doses which were scored appear negative, we know that cytotoxicity in this assay can affect the sensitivity of the test; thus, we would like to repeat this assay. Due to illness and Jim's knee surgery follow-up treatment, it will likely be the first or second week of February before he can repeat.

(3) Jim ran the surfactants, benzalkonium chloride, tallow amine, C12 alkyl sulfate, and MON59117, in the in situ micronucleus assay on 2/14/00 at the following concentrations with and without metabolic activation: benzalkonium chloride at 5000, 2000, 1000, and 500 ug/ml; tallow amine, C12 alkyl sulfate, and MON59117 at 62.5, 15.6, 3.9, 0.98, 0.24, and 0.06 ug/ml.

The benzalkonium chloride was insoluble at the top two doses (5000 & 2000 ug/ml) while there was no evidence of micronucleus induction at 1000 or 500 ug/ml. MON59117 also gave no

evidence of micronuclei induction, although it was judged toxic at 62.5 ug/ml. Both the tallow amine and the C12 alkyl sulfate produced an equivocal response for micronuclei induction. While the tallow amine was considered toxic at 62.5 and 15.6 ug/ml, the C12 alkyl sulfate didn't exhibit toxicity at any of the test doses. While both of these compounds produced a marginal response which didn't meet the test criteria for a robust positive, they did elicit an effect which was judged to be an equivocal, but test article-related effect.The negative and positve controls without and with metabolic activation demonstrated that this system was capable of detecting direct-acting and metabolism-dependent clastogens.

Todd Bunch [redacted] will be happy to discuss these results with you.
thanks for your patience,
Cindy

The Genetic Toxicology Discovery Screen Battery General Information:

Micro-Ames assay: This assay was designed to detect mutations within DNA, changes which can affect a single base pair within a gene. Typically, two different Salmonella typhimurium strains are used within the micro-Ames assay: strain TA98, which detects frameshift mutations, and strain TA100, which detects base-pair substitution mutations. Although being a scaled down version of the full Ames assay, a similar number of bacteria are exposed to the compound. Typically the highest dose tested in the micro-Ames assay is 250 μg/well versus 5,000 μg/plate used for the full Ames assay. While these differences in maximum doses are not significant when differences in surface area are considered, other factors may influence the effects observed in this screen.

In situ micronucleus Assay: The in situ micronucleus assay detects clastogenicity (or chromosomal aberrations) and inducers of non-disjunction. Both anomalies are detected by the presence of micronuclei, distinct membrane-bound nuclear material that separated from the nucleus during the cell division process. The assay is conducted by exposing Chinese hamster ovary cells in situ to compound concentrations generally up to 1,000 μg/ml. Micronuclei are scored manually using light microscopy. This assay is relatively new and a statistically based method for data analysis, incorporating both historical and concurrent control data, is currently being developed to create rigorous decision criteria (i.e., determining whether a compound produced a positive or negative response).

Advantages: These miniaturized assays are thought to be good predictors of their regulatory GLP counterparts, the Ames assay and the in vitro chromosomal aberration assay, but are shorter, higher throughput assays. Within a week, clastogenic and mutagenic information on at least 10 compounds can be obtained. They require very small amounts of compound. Assuming good compound solubility, both assays can be run with a minimum of 6 mg of compound. Both assays include metabolic activation conditions, which are important since only the metabolites of many compounds are mutagenic and/or clastogenic.

Disadvantages: Both of these assays have limited dosing schemes as well as limited historical reference information from which to compare results. The counterpart of the micro-Ames includes additional strains thereby increasing the probability of detecting weak mutagens. One counterpart of the in situ micronucleus assay is an in vivo assay; factors such as absorption, metabolism, distribution and excretion, which may affect the response, cannot be adequately predicted in the screening assay. All these caveats need to be considered when evaluating the results obtained from these screening assays.

Conclusions: The genetox screens provide valuable information to support the rank ordering of compounds. Equivocal or weakly positive results require repeat testing, while a positive result in either assay suggests the need for further testing using additional standard GLP assays, particularly if the compound is promising. Most importantly however, by screening compounds in the above assays, the likelihood that a development compound will have genetox findings after more expensive animal studies are run will be significantly reduced.