## Comments submitted by the ABTR Network

on the DRAFT FOR PUBLIC CONSULTATION - SCIENTIFIC OPINION: EFSA guidance on repeated-dose 90-day oral toxicity study on whole food/feed in rodents.

| Line | Comment |
|---|---|
| **Lines 020 - 023** | The ABTR Network members are not convinced that there is a need for a specific guideline for a 90-day rodent feeding study for novel food/GMO when OECD Test Guideline 408 has proved reliable over years and OECD do not consider this to be necessary. International harmonization of toxicity studies for the purposes of standardization and the addressing the 3Rs has been at the top of the safety testing agenda over many decades and OECD amongst a range of organizations has made huge strides in this regard. It is therefore bizarre to see EFSA produce a completely new stand-alone protocol for 90 day whole food/feed feeding studies when these have been conducted for many years by standard adaptation of OECD 408. |
| **Lines 034 -035** | This should state “animal room” not “house” unless animals can be housed in multiple |
| **Lines 053 -** | This is too strong a recommendation, it is common practise to include additional controls because of the natural variation of foods, which lead to a wider spread of results than with a single chemical substance of defined purity. This uses less animals than the |
| **Lines 149 -** | Edit “In order to provide rapidly guidance…… |
| **Lines 155 - 157** | The ABTR Network members are not convinced that there is a need for a specific guideline for a 90-day rodent feeding study for novel food/GMO when OECD Test Guideline 408 has proved reliable over years and OECD do not consider this to be necessary. International harmonization of toxicity studies for the purposes of standardization and the addressing the 3Rs has been at the top of the safety testing agenda over many decades and OECD amongst a range of organizations has made huge strides in this regard. It is therefore bizarre to see EFSA produce a completely new stand-alone protocol for 90 day whole food/feed feeding studies when these have been conducted for many years by standard adaptation of OECD 408.<br>OECD 408 is supported by a solid database of study results and internationally accepted. |
| **Lines 157 -** | Why is it indicated that "Specific attention will be paid to the development of protocols suitable for food/feed derived from GM plants" when in other parts of the document it is |
| **Lines 172 -** | This only refers to data collected from tests and seems to neglect the tiered approach embraced by EFSA including *in silico* analysis which is expected to be increasingly |
| **Lines 186 -** | Should a Guidance document refer to a specific product?? The ANSES opinion, based on using the data and study design of the MON810 study, was provided as a contribution to |
| **Lines 192 -** | This is incorrect as stated “While single chemicals and simple chemical mixtures can be administered to the test animal at dose levels which are several times higher (should read normally many times higher by up to 2 orders of magnitude) than the likely human exposure levels, this may not be possible with whole food or feed as these are bulky and can result in satiation and/or unbalanced diets if given at high levels. (Corn can be |
| **Lines 196 - 200** | The ABTR Network members are not convinced that there is a need for a specific guideline for a 90-day rodent feeding study for novel food/GMO when OECD Test Guideline 408 has proved reliable over years and OECD do not consider this to be necessary. International harmonization of toxicity studies for the purposes of standardization and the addressing the 3Rs has been at the top of the safety testing agenda over many decades and OECD amongst a range of organizations has made huge strides in this regard. It is therefore bizarre to see EFSA produce a completely new |

| | |
|---|---|
| **Lines 205 - 209** | The ABTR Network members are not convinced that there is a need for a specific guideline for a 90-day rodent feeding study for novel food/GMO when OECD Test Guideline 408 has proved reliable over years and OECD do not consider this to be necessary. International harmonization of toxicity studies for the purposes of standardization and the addressing the 3Rs has been at the top of the safety testing agenda over many decades and OECD amongst a range of organizations has made huge strides in this regard. It is therefore bizarre to see EFSA produce a completely new stand-alone protocol for 90 day whole food/feed feeding studies when these have been conducted for many years by standard adaptation of OECD 408.<br>OECD 408 is supported by a solid database of study results and internationally accepted. |
| **Lines 247-250** | While the purpose of the 90-days toxicity studies is clearly stated, it could be interpreted as advocating that these are done "rather than determining qualitative or quantitative....". Does this mean that EFSA disagrees from the tiered approach? Would 90 days studies be considered as fully replacing qualitative and quantitaive assessments |
| **Lines 266-267** | It would be usefull to understand for which cases this would be relevant. |
| **Lines 354 -** | Using this strategy which does not set out to establish a NOAEL, when the low dose shows effects (at a multiple of human exposure), will the regulators will not automatically be concerned, or third parties, that a whole food/feed has caused adverse effects? This could trigger a repeat study and more animal usage. A 2 dose level study has no foundation in science. There is no chance of developing a dose response, what happens if you join 2 dots? You get a straight line! It should either be a limit dose (single |
| **Lines 359 -** | The concepts are confusing. The first appears to relate to nutritional differences between dosage groups, eg if lipid or CHO levels vary by > 5% between dosage levels this should be adjusted back to a maximum variance of 5% otherwise one gp may get more calories than another. This is nothing to do with the incorporation of a whole food in the diet at 5% or >. As mentioned earlier corn can be added to rat diet at up to 40% and the |
| **Lines 404 -** | Is it really necessary to state specific organs? It leaves out other key organ systems. Does that mean that there is no concern for reproduction etc etc |
| **Lines 411 -** | This is the first mention of isogenic dose groups. |
| **Lines 433 -** | This and the preceding para weight mice and rats evenly but every toxicologist knows that very few studies in mice are conducted, other than acutes and mouse carcinos which are recommended to be abandoned. You cannot get enough blood etc to study properly and as this is intended to be a meaningful 90 day study maybe it should be |
| **Lines 490-503** | The document states at different places that it may be a confirmatory test (i.e., used to test specified hypotheses), and at other places that it may be an exploratory test (i.e., used to generate hypotheses). While applicants may position a study in this way, including these purposes in the guidance is confusing. Is it exploratory (research purpose?) or confirmatory? With the lack of sensitivity of the test substance (whole food), how would either of these purposes be satisfied? Also, when there are no concerns raised in other aspects of the risk assessment (especially the compositional or nutritional analysis) for a specific product, we do not see the value of a 90-day whole food study. If however, a hypothesis based scenario exists, then it should inform the |
| **Lines 500 -** | Confirmatory vs exploratory, repeating the studies to clarify hypotheses will certainly push the animal numbers up. What is the purpose of the study, the authors appear confused. Originally it was to confirm that whole foods are as safe as the traditional |
| **Lines 512 -** | The OECD 408 protocol is proven over decades by the food, pharma, agrochemical and chemical industries, where is the validation of this proposed new design via ring testing? |

| | |
|---|---|
| **Lines 523** | It will be difficult persuading toxicologists and achieving the logistics to set up a regulatory 90 day study at different times and in different animal rooms. Have the practicalities of this been considered, a contract lab approached, can it be done?…. Many of these concepts have been discussed for at least 30 years and pragmatically are seldom if ever used in regulatory toxicology except for research – this Guidance |
| **Lines 538 -** | This really is a red-herring in a publication about testing whole foods and not single substances. If the purpose is to provide a foolproof 90-day study then why do you need to spike as was done in SAFOTEST to test the sensitivity of the newly recommended |
| **Lines 557-** | This is a major problem, OECD rats are recommended to be housed 4/cage, in the same room and dosed concurrently so you will have no relevant historical control data for the 2 rat/cage block design proposal it does not exist. Randomised vs randomised block design ….does EFSA imply that OECD and all other repeat dose toxicity testing has got it |
| **Lines 587 - 588** | Confusing indications on approach for confirmatory test vs. exploratory tests. |
| **Lines 769 -** | Dose-related trends cannot be established with only 2 dose levels. |
| **Lines 812 -** | If it falls within the background why is it a change??? In which case it clearly has no health impact. A trend within background is something different. |
| **Lines 831 -** | Shouldn't EFSA also point out the other endpoints eg chronic and carcinogenicity? One cannot be selective for reproduction without explaining why: the purpose of the study is to be a sentinel study not a full blown toxicity study. Food should have reasonable |
| | EFSA's guidance should permit the use of single or multiple housing of research animals in line with the practice and experience of the toxicology testing laboratory. |
| | Other design elements that may confound interpretation include "blinding" the scientific staff and utilization of a randomized block design. According to a Best Practice Guideline published by the Society for Toxicologic Pathology there is a consensus opinion among toxicologic pathologists that implementation of a blind initial microscopic evaluation of tissues can have a negative impact on the quality of the information obtained from the study. Furthermore, the combination of a blinded staff and randomized block design may increase the potential for technicial errors during the course of the study. Since |
| | The applicant should have the option of testing at one or more dietary levels to establish dose response and calculations involving benchmark modeling if appropriate. The maximum feasible dietary incorporation rate should not cause a nutritional imbalance. This is consistent with the toxicological practice of administering a limit dose to determine margin of safety. Testing clinical blood parameters at week 7 provides little useful information since the same parameters are examined 6 weeks later at study |
| | The document places far too much emphasis on new statistical analyses and not enough on scientific interpretation. The impact of the proposed changes in statistical analysis based on power calculations appear to be marginal, at least when standard effect sizes are compared to those calculated for the existing OECD 408 experimental design. Recognising that the OECD 408 90-day rodent study is a important study in food, pharma, agrochemical and chemical testing it makes no sense to confuse experimentalists and contract labs with a truly bizarre variant protocol that attempts to redesign the wheel. Block design allowing different blocks to be treated in different animal rooms at different times with different intakes of animals and their |

| | |
|---|---|
| | The proposed guidance will lead to the use of more research animals due to study duplication. Regarding animal use, it is unclear to the applicant how many research animals will be considered acceptable for a given study using the proposed new protocol. Rather than being able to use the default number of animals outlined in OECD 408 guidelines, the applicant must conduct power calculations to estimate the appropriate number of animals taking into consideration variability of the measured parameters. However, EFSA acknowledges that accurate estimates or variability may not be available. Once the study is submitted, it may be rejected by EFSA if it is considered underpowered, or if sex-related differences are not sufficient. Since no one has any experience in what will be considered acceptable to EFSA, studies may have to be |