European Society of Toxicologic Pathology (ESTP)

Executive Committee

Letter to the editor

Serious inadequacies regarding the pathology data presented in the paper by Séralini et al. 2012.

On 19 September 2012, Food and Chemical Toxicology published online an article by Séralini et al. that reports the results of a 2-year feeding study in rats investigating the health effects of genetically modified maize NK603 with and without Roundup WeatherMAX® and Roundup® GT Plus alone.

This article attracted great attention within the scientific and regulatory community. Substantial gaps in the study design, fundamental flaws in the data analysis and erroneous interpretation of results have been pointed out by individual scientists and administration bodies. Upon request by the European Commission, the European Food and Safety Authority (EFSA) reviewed the study and concluded that the design, reporting and analysis of the study as presented in Food and Chemical Toxicology are inadequate and that this contribution is of insufficient scientific quality to be relevant in the safety assessment process (http://www.efsa.europa.eu/en/press/news/121004.htm).

The European Society of Toxicologic Pathology (ESTP) also reviewed this article. ESTP members are pathologists specialized in the diagnosis and interpretation of animal pathology from safety studies, particularly rodent carcinogenesis bioassays. Since generation and interpretation of pathology data are of paramount importance in carcinogenicity studies, the ESTP wants to highlight major pathology shortcomings in the article by Seralini et al.

First it needs to be pointed out that a conclusion of such an experimental study cannot be drawn without a good knowledge of spontaneous pathology and the availability of a robust set of internal historical pathology control data (Keenan et al., 2009). These data are especially mandatory for a proper review of this study as a limited number of control animals was included (Deschl et al., 2002; Dinse et al., 2010; Roe et al., 1995). The absence of documented knowledge of background lesions can lead to false interpretations. For instance, this is clearly the case for the Wilm's tumors reported in the study. Nephroblastomas (the internationally agreed term for this tumor entity in rodents) are well known spontaneous neoplasms in rats that occur very early in life (Frazier et al., 2012; Maxie, 2007; Mesfin, 1999; Seely, 2004). They often lead to a premature death of the animal, and in such cases the cause of death is the specific tumor and not a treatment effect. Here the comparison for the time of onset between control and treated groups is not relevant as the comparison does not apply to the same tumor type (Son et al., 2010).

The second point we would like to address is the use of atypical concepts regarding the pathology results and their presentation, for example the failure to provide tables of incidence of neoplastic data. In the paper, it is stated that not all data can be shown in one report but we have found no incidence at all in the publication. There is only one table (Table 2) in which, unfortunately, the presentation of proliferative and non-proliferative lesions is inaccurate and confusing as the data are

pooled. The decision to combine galactoceles, fibroadenomas and adenocarcinoma in the mammary gland has no scientific or regulatory justification. Those entities are completely different from each other with varied causes and have therefore to be analyzed separately. Knowing that mammary gland fibroadenomas are very common in female rats and especially in the Sprague-Dawley strain, the comparison with background incidence of each tumor type would have been more powerful to demonstrate a real treatment-related effect (Dinse et al., 2010). The sentence *"The largest palpable growths (…) were found to be in 95% of cases non-regressive tumors, and were not infectious nodules."* is very confusing. We hope that differentiating inflammatory from neoplastic lesions was not a challenge for the authors. Another clear example illustrating the lack of accuracy of the results is found in figure 3 where microscopic necrotic foci in the liver are grouped with clear-cell focus and basophilic focus with atypia. The first finding refers to a degenerative process whereas the remaining two refer to a proliferative one (Thoolen et al., 2010). Such basic error would be considered as a disqualifying mistake at an examination for pathologists. Later the tumors are said to increase in size and number but not proportionally to the treatment dose over treatment. The clinical progression of a cancer depends on the tumor type and on its biological features, but not on the dose of treatment. The presentation of chronic progressive nephropathy is inadequate: the severity should be also displayed along with the incidence for a complete understanding of a treatment-related exacerbation of this lesion (Hard and Khan, 2004). Additionally, there was erroneous interpretation of normal ultrastructural images from this carcinogenesis study: The described increased endoplasmic reticulum is the consequence of a hepatic enzyme induction by the treatment (Cheville, 1994). It has to be considered as an adaptative effect and not as an adverse effect (Capen, 2001).

The third point which we wish to highlight is the absence of reference to good practices in toxicologic pathology. A pathologist must always be responsible for the generation of histopathology data and their interpretation in these study types. He must sign to accredit this expertise. Why is the scientist responsible for the pathology assessment in the study (i.e. the Study Pathologist) not identified in the list of contributors of this work? Has there been a formal or informal peer review of the histopathological evaluation of the study? These practices are standard requirements for the evaluation of any carcinogenicity study (Crissman et al., 2004).

Last but not least we would like to comment on animal welfare issues. As most members of the ESTP are veterinarians, we were shocked by the whole body photographs of animals bearing very large tumors. When looking at the lesions, we believe those animals should have been euthanized much earlier as imposed by the European legislation on laboratory animal protection (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF). What is the added value in a scientific paper? The authors illustrate only that Sprague-Dawley rats develop mammary tumors associated with pituitary tumors. Both mammary and pituitary tumors are common background lesions in the Sprague-Dawley rat strain after 2 years (Dinse et al., 2010). But we clearly question the care brought to laboratory animals in this study which is closely associated to the quality of the produced data.

The ESTP comes to the conclusion that the pathology data presented in this paper are questionable and not correctly interpreted and displayed because they don't concur with the established protocols for interpreting rodent carcinogenicity studies and their relevance for human risk assessment. The pathology description and conclusion of this study are unprofessional. There are misinterpretations of tumors and related biological processes, misuse of diagnostic terminology; pictures are not informative and presented changes do not correspond to the narrative. We would like to finish our commentary with a question: what is the scientific rationale that led the journal reviewers and the editorial board of Food and Chemical Toxicology to accept this article for publication?

This letter presents the scientific opinion of the ESTP members represented by its Executive Committee and colleagues who did the review.

Frédéric Schorsch (Chairman of the ESTP Executive Committee), Roger Alison, Sibylle Gröters, Rudolf Müller, Anna Lanzoni, Thomas Nolte, Lars Mecklenburg (ESTP Vice Chairman), Jenny McKay (ESTP Designated Chairman), Matthias Rinke, Annette Romeike (ESTP Past Chairman).

References:

Capen, C.C., 2001. Toxic response of the endocrine system, Casarett and Doull's Toxicology: The Basic Science of Poisons. Klaassen Curtis D., p. 711.

Cheville, N.F., 1994. Ultrastructural Pathology: An Introduction to Interpretation. Iowa State University Press.

Crissman, J.W., Goodman, D.G., Hildebrandt, P.K., Maronpot, R.R., Prater, D.A., Riley, J.H., Seaman, W.J., Thake, D.C., 2004. Best practices guideline: toxicologic histopathology. Toxicol Pathol 32, 126-131.

Deschl, U., Kittel, B., Rittinghausen, S., Morawietz, G., Kohler, M., Mohr, U., Keenan, C., 2002. The value of historical control data-scientific advantages for pathologists, industry and agencies. Toxicol Pathol 30, 80-87.

Dinse, G.E., Peddada, S.D., Harris, S.F., Elmore, S.A., 2010. Comparison of NTP historical control tumor incidence rates in female Harlan Sprague Dawley and Fischer 344/N Rats. Toxicol Pathol 38, 765-775.

Frazier, K.S., Seely, J.C., Hard, G.C., Betton, G., Burnett, R., Nakatsuji, S., Nishikawa, A., Durchfeld-Meyer, B., Bube, A., 2012. Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicol Pathol 40, 14S-86S.

Hard, G.C., Khan, K.N., 2004. A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. Toxicol Pathol 32, 171-180.

Keenan, C., Elmore, S., Francke-Carroll, S., Kemp, R., Kerlin, R., Peddada, S., Pletcher, J., Rinke, M., Schmidt, S.P., Taylor, I., Wolf, D.C., 2009. Best practices for use of historical control data of proliferative rodent lesions. Toxicol Pathol 37, 679-693.

Keenan, K.P., Soper, K.A., Hertzog, P.R., Gumprecht, L.A., Smith, P.F., Mattson, B.A., Ballam, G.C., Clark, R.L., 1995. Diet, overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: II. Effects on age-related proliferative and degenerative lesions. Toxicol Pathol 23, 287-302.

Maxie, M.G., 2007. Jubb, Kennedy & Palmer's Pathology of Domestic Animals, 5 ed. Saunders Ltd.

Mesfin, G.M., 1999. Intralobar nephroblastematosis: precursor lesions of nephroblastoma in the Sprague-Dawley rat. Vet Pathol 36, 379-390.

Roe, F.J., Lee, P.N., Conybeare, G., Kelly, D., Matter, B., Prentice, D., Tobin, G., 1995. The Biosure Study: influence of composition of diet and food consumption on longevity, degenerative diseases and neoplasia in Wistar rats studied for up to 30 months post weaning. Food Chem Toxicol 33 Suppl 1, 1S-100S.

Seely, J.C., 2004. Renal Mesenchymal Tumor vs Nephroblastoma: Revisited. Journal of Toxicologic Pathology 17, 131-136.

Son, W.C., Bell, D., Taylor, I., Mowat, V., 2010. Profile of early occurring spontaneous tumors in Han Wistar rats. Toxicol Pathol 38, 292-296.

Thoolen, B., Maronpot, R.R., Harada, T., Nyska, A., Rousseaux, C., Nolte, T., Malarkey, D.E., Kaufmann, W., Kuttler, K., Deschl, U., Nakae, D., Gregson, R., Vinlove, M.P., Brix, A.E., Singh, B., Belpoggi, F., Ward, J.M., 2010. Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. Toxicol Pathol 38, 5S-81S.