LETTERS TO THE EDITOR

Carcinogenicity of Bisphenol A Revisited

To the Editor:

Munro et al. (2002) challenged my interpretation of the carcinogenesis bioassay results of bisphenol A, specifically opining that I had altered the National Toxicology Program (NTP) evaluation. In my opinion these six authors have not examined in sufficient detail the NTP Technical Report on the bioassay of bisphenol A (NTP, 1982), nor have they studied my remarks (Huff, 2001). In fact, I had gone to great lengths not to misrepresent the results or conclusions of the carcinogenicity of bisphenol A. In reality, in my letter (Huff, 2001) and in a more lengthy article (Huff 2002), I specifically used quotations liberally to adhere to the NTP results and conclusions, and not to mislead. Where I gave my experienced scientific opinion I was clear to indicate such. To clarify, and to respond to Munro et al. (2002), the following are taken from my previous letter (Huff, 2001):

In 1980 at a public peer review meeting, the NTP concluded, “Under the conditions of this bioassay, there was no convincing evidence that bisphenol A was carcinogenic for F344 rats or B6C3F1 mice of either sex” (NTP, 1982). However, the NTP also emphasized in the abstract, discussion, and conclusion that “the marginally significant increase in leukemia in male rats, along with an increase (not statistically significant) in leukemias in female rats and a marginally significant increase in the combined incidence of lymphomas and leukemias in male mice, suggests that exposure to bisphenol A may be associated with increased cancers of the hematopoietic system.

To me this clearly indicates that the NTP was emphasizing that these hematopoietic tumors may be associated with bisphenol A exposures. I went on to state that “using the current NTP levels of evidence categories (Huff, 1992) these findings would clearly be considered as ‘equivocal evidence’ or possibly ‘some evidence’ of carcinogenicity.” This I believe to be a true evaluation of the hematopoietic tumor data.

Thus, for Munro et al. (2002) to state that I deviated from the NTP bioassay report regarding these neoplastic lesions is incorrect. At that time the NTP was careful to use the phrase “no convincing evidence” in order to stress that these hematopoietic lesions may be associated with the administration of bisphenol A. One must note that the NTP did not state “not carcinogenic,” which was the vocabulary used in those days for a negative study, and that would have been used if bisphenol A did not exhibit any carcinogenic activity. Thus, Munro et al. (2002) stress the NTP conclusion of “no convincing evidence,” without, however, taking into account or acknowledging this and the other findings of note emphasized in the NTP Technical Report.

Furthermore, regarding these hematopoietic cancers, the independent Peer Review Panel instructed the NTP “to reflect the facts that leukemia in male rats showed a significant positive trend, that leukemia incidence in high-dose male rats was considered not significant only on the basis of the Bonferroni criteria [note: use of which was subsequently abandoned], that leukemia incidence was also elevated in female rats and male mice” (NTP, 1982, p. xi).

I also stated clearly in my letter:

The NTP further opined in their abstract and conclusion: “A statistically significant increase in interstitial-cell tumors of the testes in male rats was also suggestive of carcinogenesis” and continued with this caveat “but was not considered to be convincing evidence of a compound-related effect because this lesion normally occurs at a high incidence in aging F344 rats.” However, like the hematopoietic effects, this substantial and significant increase in testicular tumors in both exposure groups of male rats should likewise be considered as evidence of carcinogenicity: 35/49 (71%) in controls versus 48/50 (98%) in low dose and 46/49 (94%) in top dose (Haseman and Elwell, 1996; Huff, 2002).

Obviously, the last sentence is my opinion, based again on the phrase that the NTP stated “but was not considered to be convincing evidence of a compound-related effect.” One must note the use of “convincing evidence” to indicate again this was “not negative.” The NTP states that the “significant increase in interstitial-cell tumors of the testes in male rats was also suggestive of carcinogenesis.” What part of this does Munro et al. (2002) not understand? I believe that these testicular effects, if evaluated today, would be considered “equivocal evidence” or possibly “some evidence of carcinogenic activity,” certainly more than a negative response. Statistically, the dose-related trend was highly significant ($p = 0.001$), as were the pairwise comparisons between the control and the two exposed groups ($p = 0.001$ and $p = 0.003$, respectively).

For further comparison, only three other chemicals have been deemed related to the carcinogenicity of the testes in Fischer rats in NTP studies (controls listed first followed by exposed groups): ethylenzene TR 466 [adenoma: 36/50, (72%); 33/50, (66%); 40/50, (80%); 44/50, (88%)], isoprene TR 486 (interstitial cell adenoma, bilateral 20/50, 29/50, 37/50, 48/50; interstitial cell adenoma, including bilateral 33/50, 37/50, 44/50, 48/50), and tetrafluoroethylene TR 450 (interstitial cell adenoma: 39/50, 40/50, 48/50, 47/50). For the first two, the testicular tumor responses were included in the experimental evidence that was used by the NTP to identify these chemicals (isoprene and ethylenzene) as carcinogens, and the incidence rates were quite similar to those of bisphenol A. Importantly, the control rates for these tumors in the Fischer rat studies were 72%, 66%, and 78% compared similarly to the bisphenol A...
control rate of 71%. Therefore, for Munro et al. (2002) to base their dismissal on a “nonsignificant” survival difference is not persuasive. Their statement that “the NTP was correct in concluding that this response was not treatment related” does not reflect what is stated in the NTP Technical Report as reiterated above. That is, the NTP concluded that the increase in testicular tumors “was also suggestive of carcinogenesis,” also meaning the leukemia/lymphoma increases. Following the more recent evaluations of ethylbenzene and isoprene and testicular tumors, the NTP might do likewise with bisphenol A if it were being evaluated now.

Also in my letter I mentioned these findings:

Additional estrogenic disruptor effects from exposing rodents to bisphenol A in these bioassays include an increasing trend for tumors of the mammary glands in male rats (an unusual tumor for males), and decreased trends for tumors of the adrenal gland in male (medulla) and female (cortical) rats and for endometrial stromal polyps of the uterus in female rats.

The data for the mammary gland tumors in male rats were 0/50 controls, 0/50 in low dose group, and 4/50 (8%) in the top dose group. The dose-related trend statistic is significant at $p = 0.015$. Comparing the 0/50 controls versus the 4/50 in the top dose, the $p$-value is 0.059, but if one combines the two 0/50 groups then the $p$-value becomes significant at 0.011. Also, if one compares the historical control rates at the time the studies were done (see Haseman et al., 1990), there were 51/1936 control male rats with tumors of the mammary glands; comparing this 2.6% (1.3 animals per group of 50) versus the 8% seen with bisphenol A gives a $p$-value of 0.047, a marginally significant increase. Furthermore, the 8% incidence with bisphenol A is 3.1 times the average historical control rate, a rate that would be of concern if found in humans.

Importantly, male rats are considered particularly non-responsive to chemicals that cause mammary tumors (Dunnick et al., 1995; Wolff et al., 1996; Huff, 2000) Only seven chemicals out of 500 (1.4%) tested by the NTP have caused tumors of the mammary gland in male rats whereas 44 of these 500 (8.8%) have caused tumors of the mammary gland overall, mainly in female rats (37 chemicals) and less so in female mice (12 chemicals), with none in male mice (http://ntp-server.niehs.nih.gov/htdocs/Sites/MAMM.html; Huff et al., 1991). Thus, in my opinion these endocrine tumors should be considered as related to the administration of bisphenol A.

Another interesting finding in male rats is for total malignant tumors; 19 were found in control animals, 25 in the low dose group, and 33 in the high dose group. This is a reasonable and fine dose-response trend. These data are typically not used by the NTP as evidence of carcinogenicity but are used routinely, and in my opinion appropriately, by the Ramazzini Foundation (Soffritti et al., 2002). Statistically, there is a positive dose response trend for animals with malignant tumors ($p = 0.028$: 18/50 versus 22/50 and 28/50) as well as for total malignant tumors per animal ($p = 0.014$: 19/50 versus 25/50 and 33/50).

I ended my letter (Huff, 2001) and longer article on bisphenol A (Huff, 2002) with:

Overall, it appears that BPA exposure via the diet for two years should be considered associated with tumors of the hematopoietic system in rats and mice, and of the testes and of the mammary glands in male rats. Marginal decreases in tumors of the adrenal gland and of the uterus in rats should also be considered chemically related.

In my opinion, the data (three tumor sites in male rats plus total tumors, with one each for female rats and male mice) support this carcinogenesis and do not stray substantially from that detailed and intended in the NTP Technical Report. I stand by the scientific and primary prevention interpretations offered previously and reiterated herein. The particular lessons here are that the use of the terms “no convincing evidence” does not mean and should not be construed as a “negative response,” and one must evaluate the totality of the experimental findings (Huff, 1999; Tomatis et al., 2001), because there are several in this bioassay of bisphenol A that are biologically meaningful.

James Huff

References


To the Editor:

The issue of carcinogenicity of bisphenol A was discussed in previous letters to the editor (Huff, 2001; Munro et al., 2002). In his letter revisiting the subject, we note that Huff (2002) does not present any new information that was not previously addressed. As we have already commented on the NTP findings in our earlier letter (Munro et al., 2002), we have chosen to present additional information in support of our findings with regard to the lack of carcinogenic potential of bisphenol A. However, we would first like to indicate that our original response (Munro et al., 2002) was not meant to personally challenge Huff (2001) but merely to point out the final concluding statement of the NTP (1982) report on BPA that specifies that “under the conditions of this bioassay, there was no convincing evidence that bisphenol A was carcinogenic for F344 rats or B6C3F1 mice of either sex.” The discussion of the NTP report does state, as indicated by Huff (2001, 2002):

When the marginally significant increase of leukemias in male rats and the combined incidence of lymphomas and leukemias in male mice are considered along with the increase in leukemia incidence (not significant) in female rats, the evidence is suggestive of a carcinogenic effect on the hematopoietic system.

However, the NTP (1982) prefaches that statement with the following:

Leukemias in male rats occurred at an incidence that showed a statistically significant positive association with the dose of bisphenol A. Although the incidence in high-dose male rats appeared to be statistically significant (p = 0.030), it did not meet the Bonferroni inequality criterion of p = 0.025 for comparing the dosed groups with a common control. The incidence of leukemias was also increased in female rats, but the observed increases were not statistically significant. Life table analyses, adjusted for intercurrent mortality, were also carried out for the leukemia data. It was found that, for male rats, neither the high-dose effect nor the dose-response trend was statistically significant (p = 0.141 and p = 0.074). The female rats likewise showed no significant effects. The increased incidence of leukemia in rats was therefore not considered to be convincing evidence of carcinogenicity in rats.

Consistent with our own review (Haighton et al., 2002) and earlier response (Munro et al., 2002), we would like to point out the concurring opinion of the European Commission’s Scientific Committee on Food concerning bisphenol A with regard to carcinogenicity and genotoxicity, which was recently made public (SCF, 2002). The SCF states that “there was no evidence of substance-related carcinogenicity in 2-year rat and mouse bioassays using dietary administration (NTP, 1982). A similar conclusion was reached in other recent reviews” (the SCF cites the German Chemical Society, 1997; Dutch Expert Committee on Occupational Standards, 1996; Veenstra and Webb, 1999; and EU, 2001). The SCF (2002) also states that:

BPA has been tested extensively for genotoxicity, both in vitro and in vivo. The majority of in vitro studies, which included tests for gene mutations in bacteria, chromosomal aberrations in cultured mammalian cells and gene mutations in cultured mammalian cells, were clearly negative, with a few producing equivocal results that were not replicated in other studies using the same test system. In vivo studies, including a mouse micronucleus test conducted to modern standards and a rat dominant lethal study, were negative. Low levels of DNA adduct formation were observed in vitro and in vivo but the covalent binding index was only 0.01. In vitro tests for aneuploidy were positive at doses close to or causing cytotoxicity, but the absence of micronuclei in the in vivo mouse micronucleus test provides some reassurance that the aneugenic potential observed in vitro is not expressed in vivo. Recent extensive reviews have all concluded that BPA is non-genotoxic in vivo (German Chemical Society, 1997; Dutch Expert Committee on Occupational Standards, 1996; Veenstra and Webb, 1999; EU, 2001).

Likewise, the Scientific Committee on Toxicology, Ecotoxicology and the Environment (CSTEE, 2002), a group of distinguished scientists that provides an independent evaluation of risk assessments completed by the European Commission Rapporteur, “agrees with the overall conclusion that bisphenol A has no significant mutagenic potential in vivo” and “that bisphenol A does not have a significant carcinogenic potential.”

In addition, we wish to reiterate that human exposure to BPA is considerably lower than the exposure experienced in the NTP cancer bioassays. Based on UK food survey data, the SCF identified a mean BPA level in foods of about 20 micrograms/kg food or approximately 0.02 ppm. In the NTP (1982) study, rats were fed diets containing 1000 or 2000 ppm BPA, male mice were fed diets containing 1000 or 5000 ppm BPA, and female mice were fed diets containing 5000 or 10,000 ppm. On a body weight basis, 0.02 ppm in food corresponds to approximately 0.00037 mg/kg body weight/day for 60-kg adults and 0.00085 mg/kg body weight/day for 8.8-kg infants (based on 97.5 percentile consumers of canned foods [SCF, 2002]). The consumption estimate is representative of a worst-case scenario. On a weight basis, the intake from the NTP (1982) study is approximately the following: 74 or 148 mg/kg body weight/day (male rats); 74 or 135 mg/kg body weight/day (female rats); 120 or 600 mg/kg body weight/day (male mice); and, 650 or 1300 mg/kg body weight/day (female mice). The doses used in the toxicology studies are between 87,000 and 1,500,000 times greater than the estimated BPA intake for infants at the 97.5 percentile consumption level. This vast magnitude of difference and the lack of convincing evidence of carcinogenic effects in rats and mice, support de minimis risk for humans.
The lack of convincing evidence of carcinogenicity in the rodent bioassays, combined with the lack of genotoxicity/mutagenicity activity, and the fact that metabolism data indicate that BPA is rapidly glucuronidated and excreted, in addition to the trivial exposure for humans, supports our conclusion, and those of EU scientific committees, that BPA is not a carcinogenic risk to humans.

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REFERENCES


