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Inflammation: More Than One Explanation

I read with interest the *EHP* supplement on oxygen radicals and lung injury (vol. 102, supplement 10). I would like to take this opportunity to comment about this supplement and raise a key issue concerning the major concepts regarding the mechanisms of cellular injury in inflammatory diseases.

As an active investigator in this field of research, I cannot fully understand why there was no mention in the supplement about the basic understanding that cellular damage in inflammation is multifactorial. The nonexpert reader of this supplement might receive an erroneous impression that oxygen radicals, per se, are the exclusive toxic agonists that induce cellular injury. Many in this field share the view that cellular damage in inflammatory diseases might be caused by a "coordinated cross-talk" among oxidants, membrane-damaging agents, proteinases, arachidonic acid metabolites, phospholipases, cationic proteins, and cytokines. All these agents are likely to be present in sites of infection and inflammation. But sadly, none of the publications elaborating on this multifactorial view are quoted in modern textbooks or in symposia on inflammation and inflammatory diseases. Instead, the literature is filled with publications that insist on a single agonist, be it an oxidant, a protease, a cytokine, etc., in experimental models. No attempt to integrate the various agonists into the full picture is made.

Several of our publications (*1–7*) deal with synergistic interactions among multiple proinflammatory agonists in cellular injury during inflammation. I believe that this issue is important, timely, and might contribute to an understanding of how drugs, chemicals, and xenobiotics function *in vivo*.

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Response

We appreciate the interest shown by Dr. Ginsburg in our recent conference proceedings (*EHP* 102, supplement 10). As stated in the preface of those proceedings, The Oxygen Radicals and Lung Injury Conference was the first of its kind dedicated to pulmonary science. Therefore, in this conference, the primary attempt was to focus on oxygen radicals and their involvement in toxic insults and the ensuing pathophysiological processes in the lung. We did not ignore the importance of multifactorial relationships of other cellular reactions and products involved in cellular damage and injury. In fact, these issues were addressed in the presentations of Ward (*1*), Holian et al. (*2*), Repine (*3*), Torphy et al. (*4*), and Demers and Kuhn (*5*). The complex network of micromolecular reactions have not been fully defined to understand the coordination, modulation, and integration of cellular functions. In many pulmonary diseases (e.g., cancer, emphysema, pneumoconiosis) in which oxygen radicals are implicated, the disease becomes evident only after several years. Subtle damage or changes to biomolecules and their relationships to the coordination and interactions of oxygen radical generation and degradation are important issues to be dealt with in greater detail to understand the synergistic concepts of lung dieases. We hope that future conferences will address these and other issues.

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MTBE: Not Carcinogenic

Subsequent to publication of *EHP*'s timely article on the toxicological potential of methyl-*tert*-butyl ether (MTBE; vol. 103, pp. 666–670), the long-awaited study from the Ramazzini Foundation of Oncology and Environmental Sciences appeared in print (*1*). This was a landmark publication because for months we in the scientific community had been advised that the data predicted dire health hazards for humans exposed

to MTBE. According to the *EHP* article, several scientists held the hope that the "Maltoni" work would clarify questions about MTBE's carcinogenic potential.

Despite the enthusiasm of some scientists for Belpoggi's results, the most cursory examination of the paper reveals critical issues that show the data have been grossly overinterpreted. Rather than predicting a health hazard, the data indicate that rats tolerate enormous daily oral doses of MTBE without exhibiting evidence of either tumor or nontumor pathology.

Leydig cell tumors in high-dose male rats. According to Belpoggi et al., the administration of MTBE as an olive oil gavage to male rats (1.0 g/kg, 4 days per week) was associated with a significant increase in the incidence of Leydig cell tumors. But examination of the total information in the paper shows that the reported effect cannot be attributed to MTBE. The apparent association was due to a survival differential between control and dosed animals. Male rats administered the highest dose of MTBE survived longer than the control group. It is well known that Leydig cell tumor incidence is age related. The longer a rat survives, the more likely it is to have Leydig cell tumors. Claiming this survival-related effect to be indicative of a human health hazard strains the bounds of scientific logic. This is particularly true since Leydig cell neoplasms are most likely unique to rats and appear to have no predictive utility for human carcinogenic responses (*2*).

Lymphoma and leukemia (combined) in female rats. Belpoggi et al. reported that MTBE increased the incidence of lymphomas and leukemias (combined) in female rats. Since no mention was made of the incidences of these neoplasms individually, one can only assume that neither was significantly elevated.

The scientific validity of combining lymphomas and leukemias for statistical purposes is highly questionable. A National Toxicology Program working committee reviewed scientific guidelines and criteria for the combination of neoplasms during the interpretation of rodent carcinogenesis studies (*3*). According to that group of experienced pathologists, combining certain tumors for statistical purposes is appropriate and might afford enhanced insights into the biological effects of the test chemical. In other cases, however, combinations are unjustifiable and can lead to overestimates of carcinogenic potential.

According to the NTP panel, it is reasonable to combine different types of leukemias and to combine different types of lymphomas. But it is not appropriate to combine leukemias with lymphomas. Treatment-associated increases in the incidence of one or the other of these tumors of diverse cellular origin may be suggestive of an oncogenic effect. But, since the incidence of neither was significantly and independently elevated, the authors' interpretation of this portion of the study represents an overestimation of carcinogenic potential.

The science of carcinogenic hazard identification and risk assessment has progressed well beyond the days of simply counting tumors and then making grand leaps to unfounded and insupportable conclusions. As we expand our understanding of chemical carcinogenesis and the predictive validity of our experimental models, we must employ critical and scientific thought processes that incorporate the total knowledge about the chemical. The total of pertinent knowledge about the carcinogenic effects of MTBE in laboratory animals shows that:

- The oral administration of up to 1 g/kg of MTBE four days a week produced neither neoplastic nor non-neoplastic changes in male and female rats,
- The chronic inhalational administration of grossly toxic concentrations of MTBE produced an increased incidence of hepatocellular adenomas in female (but not male) mice and an increased incidence of renal tubular cell adenomas and carcinomas (combined) in male (but not female) rats,
- Neither MTBE nor its metabolite, tertiary butyl alcohol, possess genotoxic potential in either *in vitro* or *in vivo* models,
- A potential metabolite of MTBE, formaldehyde, possesses equivocal genotoxic potential in mammalian models, but
- Even when administered at inhalational doses that are lethal to rats and cytotoxic to mice, MTBE possesses no genotoxic potential in *in vivo* mammalian models.

These scientific facts lead to the conclusions that supramaximal inhalational doses of MTBE cause increased incidences of liver neoplasms in female mice and renal neoplasms in male rats. But since MTBE and its metabolites possess no genotoxic potential, the proliferative changes in response to toxic doses are mediated through nongenotoxic mechanisms that require cytotoxicity to precipitate proliferation. Because of the intense odor (and taste) of MTBE, humans will not tolerate either air or water concentrations sufficient to produce the cytotoxic precursors required to promote cellular proliferation. In short, the carcinogenic hazard associated with MTBE has been identified and defined. The human risk, however, appears to be so small that it is essentially nonexistent.

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Clarification: Chemical Syngergism

In a recent *EHP* Forum article ("Menace in the Mix," vol. 103, pp. 792–793) concerning Dr. Mohammed Abou-Donia's work related to the Gulf War syndrome, I am quoted as saying first "It's a plausible hypothesis that synergism occurred" and second "That's not to say [the hypothesis] is an appropriate lead for further investigation." Clearly these two statements are contradictory, and the second tends to place me in a adversarial position relative to Abou-Donia. All of this arose from a background discussion of synergism, of the nature of hypotheses and how science proceeds, not from a specific discussion of Abou-Donia's work, since the latter was not available to me.

My position, based on the preliminary statements that have appeared concerning this work, is that it is interesting, that it provides a plausible hypothesis, and that it does indeed provide a basis for further studies. I hope, and believe, that nothing I said in the interview was critical of the authors of this work and did not go beyond what I would have discussed with them in a friendly discussion between fellow toxicologists.

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