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Human Exposure to Selected Animal Neurocarcinogens: A Biomarker-Based Assessment and Implications for Brain Tumor Epidemiology

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Abstract

This review is based on the proceedings from the Second Lebow Conference held in Chicago in 2007. The conference concentrated on developing a framework for innovative studies in the epidemiology of environmental exposures, focusing specifically on the potential relationship with brain tumors. Researchers with different perspectives, including toxicology, pharmacokinetics,

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and epidemiological exposure assessment, exchanged information and ideas on the use of biomarkers of exposure in molecular epidemiology studies and summarized the current knowledge on methods and approaches for biomarker-based exposure assessment. This report presents the state of science regarding biomarker-based exposure assessment of the 4 most common neurocarcinogens: acrylamide, 1,3-butadiene, N-nitroso compounds, and polycyclic aromatic hydrocarbons. Importantly, these chemicals are also carcinogenic in other organs; therefore, this discussion is useful for environmental epidemiologists studying all cancer types.

Keywords

Brain Tumors; Biomarkers; Acrylamide; 1,3-Butadiene; N-nitroso Compounds; Polycyclic Aromatic Hydrocarbons; Epidemiology

Introduction

Epidemiology of malignant brain tumors provides very few clues on their etiological factors (Fisher et al., 2007; Wrensch et al., 2005). Other than high-dose radiation, there are no established environmental risk factors for brain tumors. Interestingly, except for rare mutations (which account for a small % of cases), no genetic risk factors have been identified either. Therefore, our attention was focused on investigating whether known animal neurocarcinogens are associated with a risk for brain tumors in humans.

In animal studies, brain tumors are induced by various chemicals. The National Toxicology Program (NTP) tested many chemicals, of which 13 have been associated with site-specific tumor induction in brain in animal models (NTP,.2007). We evaluated the results of these experiments based on two most frequently used criteria – statistical significance of association and dose-response relationships. For 1,3-butadiene, glycidol, and procarbazine hydrochloride, the association with brain tumor incidence is very strong, with a statistically significant dose-response. The strengths of association for bromoethane, chloroethane, C.I. Direct Blue 15, 3,3′-dimethoxybenzidine dihydrochloride, 3,3′-dimethylbenzidine dihydrochloride, furosemide, and isoprene are not as strong as for 1,3-butadiene, procarbazine hydrochloride, and glycidol. Further, for 1,2,3-benzotriazole, diphenydramine hydrochloride, and divinylbenzene, the association is weak, with no statistically significant change in brain tumor incidence or decrease in latency or survival, and no dose-response relationship. In addition, our review of the toxicity literature identified several other industrial compounds (ethylene oxide, acrylamide, acrylonitrile, propane sultone, propylene imine) and chemotherapeutic agents (N-nitroso compounds and cyclophosphamide) as animal neurocarcinogens (Sills *et al.*, 1999). These animal carcinogenicity data provide central evidence for the hypothesis that chemical exposures may also be one of the causal factors for brain tumors in humans.

Although scientific literature on chemical exposures in the environment is expanding, current evidence for the epidemiological link between environmental exposures and brain cancer is inconclusive. Traditional epidemiological studies relied on occupational history as the basis of exposure information to chemicals. However, occupational studies do not provide definitive evidence either for or against the involvement of chemical exposures in the etiology of brain tumors. Methodological limitations of occupational studies may explain these inconsistencies. Occupational case-control studies assess exposure to chemicals based on self-report data of occupational histories and of certain specific exposures. Even with the construction of job-exposure matrices and use of expert assessment, such questionnaire-based data provide a variable accuracy and low sensitivity for qualitative and quantitative exposure assessment. In addition, these methods are limited in addressing the complexity of

multiple and overlapping exposures in the workplace. Occupational cohort studies have more instruments in their arsenal of exposure assessment, such as objective hygiene measurements of external dose or models for exposure determinants that allow prediction of external dose. However, occupational cohorts have two major limitations. First, the exposure range in occupational cohorts is not as wide as in communities. Second and most important, the number of brain tumor cases is not sufficient to conduct a precise statistical analysis. Even less so can histological diversity of brain tumors be addressed by occupational cohort studies. Conventionally seen as a multiplicity of etiological pathways, specific histologies may be associated with different neurocarcinogens; thus, not differentiating between the histological subtypes of brain tumors may lead to underestimation of their associations with neurocarcinogens. Finally, occupation-based exposure assessment does not take into account other sources of exposure, such as diet and the general environment. Thus, a significant barrier to advancing our understanding of environmental causes of brain cancer is lack of valid and precise exposure measurements that can quantify low-level chronic exposures pertinent to diseases that have a long latent period, such as cancer. However, with the advances in analytical chemistry, it is now possible to supplement and validate exposure scores derived from questionnaires by using biomarker measures, which are rapidly evolving. Biomarkers of animal neurocarcinogens may be a beneficial first step in understanding the variability in internal human exposures. This could help to further the study of the environmental etiology of brain tumors.

This review is based on the proceedings of the Second Lebow Conference held in Chicago, Illinois, in March 2007. This meeting provided a forum for experts in brain tumors from neuropathology, clinical and basic sciences, and epidemiology. The Second Lebow Conference focused on developing a framework for innovative studies in the epidemiology of environmental exposures and brain tumors through exchange of information and ideas between researchers with different perspectives, including toxicology, pharmacokinetics, and epidemiological exposure assessment. It is not our intention to present a comprehensive review of issues surrounding the exposure of humans to neurocarcinogens in the environment. Our primary objective was to (1) review the use of biomarkers of exposure in molecular epidemiology studies and (2) better understand the current knowledge on methods and approaches for biomarker-based assessment of exposure to neurocarcinogens. Specifically, the state of science regarding biomarker-based exposure assessment of 4 neurocarcinogens: acrylamide, 1,3-butadiene, *N*-nitroso compounds, and polycyclic aromatic hydrocarbons (PAH) is reviewed. These 4 neurocarcinogens were selected because they are most widely spread in the environment and often found in the workplace.

Use of Biomarkers to Measure Exposure

In biomarker assessment, the goal is to measure biological, biochemical, or molecular alterations that may influence the risk of disease. Biomarkers are classified as biomarkers of exposure, effect, and susceptibility. Biomarkers of exposure may measure internal dose or biologically effective dose. While the former provides a measure of the amount of a potential carcinogen or its metabolites in cells, tissues, and body fluids (such as saliva, blood, urine, and feces), the latter measures the amount of carcinogen that has reacted with critical cellular macromolecules (i.e., DNA) or an established surrogate target (i.e., proteins in blood). Biomarkers of effect provide a measure of early responses of cells to carcinogen-induced damage and may include DNA single-strand breaks (SSB), DNA adducts, chromosomal aberrations (CA), sister chromatid exchanges (SCE), micronuclei (MN), and somatic mutations. Biomarkers of susceptibility identify (1) differences in the body's metabolism of carcinogenic chemicals (uptake, activation, and detoxification); (2) DNA repair; (3) inherited or acquired alterations or polymorphisms in proto-oncogenes, tumor suppressor genes, DNA repair genes, or metabolizing genes; (4) nutritional status; and (5)

hormonal and immunological factors. Although biomarkers of effect and susceptibility concern with different type of measurements, they are related to each other. Finally, when biomarkers are not available or can not be measured, the data on functional genetic polymorphisms can be useful. Such polymorphisms should be related to the biological pathways affecting the effect of the exposure. If these polymorphisms are known, they can help in assessing the potential for the harmful influence of the exposure, thus, providing more insight than the exposure data alone.

Using internal exposure information gleaned from different biomarkers is an improvement over traditional methods for assessing human exposures; however, there are important pitfalls. First, extracting chemical-specific data from biomarker studies may not always be possible, especially when biomarkers of effect and susceptibility are targeted. Second, biomarkers of internal dose present integrated indices of exposure over a certain period of time (Vainio,.1998). Therefore, such internal dose measurements may be valid to assess steady-state exposures, but not lifetime exposures. This report will consider how these issues, outlined in detail with respect to general epidemiology (Vineis and Perera, 2007), relate to the assessment of the 4 neurocarcinogens and carcinogen groups discussed below.

Neurocarcinogens Prevalent in the Human Environment and Their Biomarkers

Acrylamide

Acrylamide (AA) is an important industrial monomer that has been used since the 1950s to manufacture water-soluble polymers (polyacrylamide) (IARC,.1994). The recent discovery of AA in a variety of fried and baked foods (Tareke *et al.*, 2002) and coffee raised public health concerns, because AA was classified as a probable human carcinogen on the basis of findings of rodent carcinogenicity studies (IARC, 1994; Bull et al., 1984a; 1984b; Friedman et al., 1995; Johnson et al., 1986).

Once absorbed by the body, AA readily passes through biological membranes as a result of its low molecular weight and high water solubility. AA is metabolized by P450 2E1 to the epoxide glycidamide (GA) (Sumner *et al.*, 1999). Both AA and GA are susceptible to nucleophilic attack. Reaction of both compounds with glutathione represents a detoxification pathway and results in urinary excretion of mercapturic acid conjugates. AA and GA bind to plasma proteins such as Hb, as well as interact with DNA to form DNA adducts. However, GA is far more reactive with DNA than AA, and predominantly produces the DNA adduct N7-GA-Gua. Minor adducts include N1-GA-Ade and N3-GA-Ade (Segerback et al., 1995; Gamboa da et al., 2003). Multiple lines of evidence indicate that GA is responsible for AA-induced genotoxicity: (1) GA exhibits significantly higher reactivity with DNA than AA, (2) GA-DNA adducts are found in all tissues tested, (3) GA-DNA adducts accumulate with repeated dosing, (4) GA exhibits greater genotoxic potency in in vitro and in vivo studies, and (5) GA has structural similarity with ethylene oxide and glycidol, which are known carcinogens (Besaratinia and Pfeifer, 2007; Doerge et al., 2005a; 2005a; Exon,.2006; Favor and Shelby, 2005; Ghanayem et al., 2005a; 2005b; 2005c).

Two animal studies showed that chronic exposure to AA was associated with increased incidences of tumors at different sites, including central nervous system (CNS) (Friedman. et al., 1995; Johnson. et al., 1986). Although in the earlier study glial tumors of the CNS were more frequent in AA-exposed rats (Johnson. et al., 1986), this outcome was not confirmed in the later study (Friedman. et al., 1995). However, due to several shortcomings (Rice,.2005), this later study remains inconclusive. An important indication of AA neurocarcinogenicity is increased DNA damage and GA-DNA adducts in brain tissue of rodents exposed to AA (Segerback. et al., 1995; Maniere et al., 2005).

The only known adverse effect of AA in humans is neurotoxicity resulting from short-term exposure to high levels of the compound in industrial accidents (Exon, 2006). Epidemiological studies of dietary exposures yielded inconclusive results thus far (Rice, 2005). Multiple studies of two groups of workers exposed to AA revealed no change in brain tumor risk in exposed workers. Collins et al. (1989) found similar standardized mortality ratios (SMRs) of 0.45 for the concentrations of AA <0.001 mg/m^3-year and 0.42 for ≥0.001 mg/m^3-year (confidence intervals were not published). The later analysis of this cohort published by Marsh et al. (1999) found SMRs 0.64 (95% CI 0.32–1.14) for <0.001 mg/m^3-year and 0.74 (95% CI 0.15–2.15) for ≥0.001 mg/m^3-year; these findings did not change meaningfully in the updated analysis conducted by Marsh et al. (2007): SMRs were 0.73 (95% CI 0.41–1.21) for <0.001 mg/m^3-year and 0.58 (95% CI 0.12–1.69) for 0.001 mg/m^3-year. Two other studies of workers in the acrylamide manufacturing industry indicated no cases of brain or CNS tumor (Sobel et al., 1986; Swaen et al., 2007).

Biomarkers used to determine exposure and internal dose of AA have been reviewed (Dybing et al., 2005; Doerge et al., 2007). Potentially useful biomarkers include Hb adducts, DNA adducts, and urinary metabolites. Measurement of Hb adducts is well established for the estimation of internal exposure to AA and GA in humans. AA-Hb may used as a biomarker for average AA exposure, while GA-Hb may utilized as a biomarker for the internal GA dose and the genotoxic impact (Bjellaas *et al.*, 2007a). The measurement of AA- and GA-Hb adducts was used to determine exposure to AA in the occupational environment and from smoking (Ogawa *et al.*, 2006). However, Hb adduct levels have poorly correlated with estimated dietary exposures (Wirfalt et al., 2008; Hagmar et al., 2005; Kutting et al., 2005), possibly due to the difficulty in obtaining accurate exposure data from food questionnaires. GA-DNA adducts might be used as markers of the biologically active dose of GA that reaches the DNA (Dybing. et al., 2005). Further, according to toxicokinetic studies in rats and mice (Doerge. et al., 2005a; 2005b; 2005c; Tareke et al., 2006) the level of GA-Hb adducts in blood, an easily accessible biomarker, may be used to predict tissue DNA-adduct levels. Urinary biomarkers provide a readily accessible means for evaluating the detoxification of AA and its conversion to GA (Doerge. et al., 2007) and are primarily dependent on recent dietary intake of AA (Vesper et al., 2005; Boettcher et al., 2006; Bjellaas et al., 2007b). To investigate urinary biomarkers as a measure of internal exposure to AA, Doerge et al. (2007) measured 24-hr urinary metabolites, including free AA and GA and their mercapturic acid conjugates (AAMA and GAMA, respectively). They found significant linear correlations between urinary levels of AA and AAMA, as well as those of GA and GAMA. However, despite statistical significance, considerable interanimal variability was observed in all urinary measurements. In contrast, Hb adducts represent an average exposure over a period of about 4 months. Therefore, urinary biomarkers are much more sensitive to short-term changes in the diet than Hb adducts.

Based on these studies, Young et al. (2007) developed a pharmacokinetic model for AA, GA and urinary metabolites of AA and GA to predict the potential DNA adduct levels in humans resulting from dietary exposure to AA. The results suggest that DNA adduct formation is linear across most dietary human exposure levels. The steady-state human liver GA-adduct level from exposure to background levels of AA in the diet was calculated to be between 0.06 and 0.26 adducts per 10^8 nucleotides. Such models may be useful in predicting effects of chronic low-dose exposure to AA in humans, particularly because steady-state DNA adduct levels have correlated directly with tumor incidence in some rodent studies. Ultimately, the question of whether this type of exposure will result in human health problems remains unanswered. Thulesius and Waddell (2004) suggested that the level of exposure from dietary routes (approximately 0.4–0.5 μg/kg per day) is too low to be a concern. However, the Joint FAO/WHO Expert Committee on Food Additives in 2005 recommended continued chronic studies to evaluate AA for carcinogenicity and

neurotoxicity and further toxicological/pharmacological modeling efforts to determine biomarkers of AA exposure, along with attempts to lower AA in food (Exon, 2006; WHO, 2006).

1,3-Butadiene

1,3-Butadiene is a synthetic monomer used in the production of synthetic rubber. This compound is found in many occupational settings, such as the rubber, transportation, waste treatment, and petroleum industries, including the manufacture of petroleum-based products (Morrow, 2001). In addition, 1,3-butadiene is found ubiquitously in the environment. Individuals may be exposed to low levels of 1,3-butadiene through contact with smoke (tobacco, fire, cooking with an open flame, etc.); emissions from coal, wood, oil, or waste burning; emissions from vehicles or other gasoline/diesel-powered engines; and exposure to other petroleum-based products, such as kerosene, heating oil, and roofing tar. The primary route of exposure to 1,3-butadiene is inhalation.

To become carcinogenic, 1,3-butadiene needs to be metabolized. The metabolism of 1,3-butadiene is complex, and important species differences exist (Himmelstein *et al.*, 1997). Genes involved in the metabolism of 1,3-butadiene include the class I metabolism gene CYP2E1 and class II detoxifying genes in the glutathione pathway, as well as the epoxide hydrolases. The major reactive metabolite formed is the diolepoxide, while the monoepoxide and diepoxide are each formed to a lesser extent. The diepoxide, diepoxybutane, is 100–200-fold more mutagenic than either the mono- or diolepoxides (Cochrane and Skopek, 1994).

1,3-Butadiene is classified as a known human carcinogen by EPA, NTP, and IARC. Evidence for its carcinogenicity arises from studies of workers from the styrene-butadiene rubber industry. Occupational studies in the rubber industry found increased incidence of leukemia (Delzell *et al.*, 2006) and lymphosarcoma (Divine and Hartman, 2001). A variety of tumors, including brain tumors, are found in animal models exposed to 1,3-butadiene (Kim *et al.*, 2005), which supports both its carcinogenicity and potential for being a neurocarcinogen. Mice exposed to high (625-ppm) levels of 1,3-butadiene for at least 13 weeks have an increased risk of malignant glioma (NTP,.1993). The morphology of these tumors is similar to that of human brain tumors.

Numerous adducts formed by 1,3-butadiene metabolites in DNA and Hb may be used as biomarkers. N7-guanine adducts are the most prevalent DNA adducts; in addition, adenosine adducts were detected. Hemoglobin adducts include N-(2,3,4-trihydroxybutyl)valine (THB-val), the most prevalent adduct, and lesser amounts of N-(2-hydroxy-3-butenyl)valine (HB-val) and N,N-(2,3-dihydroxy-1,4-butadiyl)-valine (pyr-val). The pyr-val Hb adduct reflects formation of the mutagenic diepoxide, presenting a biomarker of the internal dose of a toxic metabolite. In humans, THB-val was shown to be a reliable indicator of exposure to 1,3-butadiene (Hayes et al., 2000; Albertini et al., 2007). At current occupational exposures, however, pyr-val was not detectable in exposed human subjects (Swenberg *et al.*, 2007). New instrumentation and methodology have recently been developed that can detect pyr-val in exposed workers and in nonexposed individuals, which suggests that there are environmental sources of exposure other than occupations (Swenberg et al., unpublished data).

Several genotoxic effects were studied in relation to occupational exposure, specifically, SCE frequency of somatic mutations measured by hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) assay, as well as aneuploidy. Mutagenicity may result from G=G cross-links that are created after 1,3-butadiene exposure (Goggin *et al.*, 2007). Accumulation of the p53 protein, loss of heterozygosity of the p53 and Ink4a/Arf genes (Kim. et al., 2005), and G→A transition mutations in the p53 gene were all observed in

malignant gliomas in mice exposed to 1,3-butadiene. These changes are similar to changes observed in human glial tumors (Kleihues *et al.*, 2000), and the G→A transition is compatible with the G=G cross-links induced by 1,3-butadiene. However, studies provided no evidence for a genotoxic effect in those individuals exposed to current occupational levels. Because GSTT1 affects 1,3-butadiene metabolism, subjects were stratified by GSTT1 polymorphism. No evidence of an effect was found by GSTT1-genotype. In contrast, in vitro exposure of lymphocytes from GSTT1-null individuals to 1,3-butadiene diepoxide did result in an increased level of SCE, suggesting that this detoxification pathway is important for in vitro exposures, but that such exposures do not occur under present-day occupational settings. A subsequent study in humans also showed no significant difference in genotoxic effects between controls and cases exposed to current occupational levels of 1,3-butadiene (Albertini *et al.*, 2003). However, it remains unknown if the high occupational exposures in the 1940–1970s (Morrow, 2001), which were up to 500-fold higher than current occupational standards, might have induced genetic changes.

While previous studies primarily utilized THB-val as the biomarker of choice, new technology that allows detection of previously undetectable levels of pyr-val, the Hb adduct representing the more mutagenic diepoxide, will increase our knowledge of the relationship between 1,3-butadiene exposures and cancer risk. Future studies will need to investigate the metabolism, as well as exposure-response relationships, of 1,3-butadiene in humans. Further consideration of the genes involved in the metabolism of 1,3-butadiene and formation of pyr-val globin adducts may assist in interpreting epidemiologic studies to determine any causal relationship between brain tumors and 1,3-butadiene exposure.

N-nitroso compounds

N-nitroso compounds can be divided into two classes – nitrosamines and nitrosamides, the latter class comprising the nitrosoureas. Human exposure to *N*-nitroso compounds occurs through many different exposure routes: dietary (72%), occupational (25%), cigarette smoking (2%), and miscellaneous sources including cosmetics, pharmaceuticals, and indoor and outdoor air (1%) (Tannenbaum,.1983; Tricker,.1997). *N*-nitroso compounds are formed by nitrosation reactions between secondary and tertiary amines and nitrite or other nitrosating agents. These reactions occur in the environment (exogenous exposure) and in the body (endogenous exposure). Endogenous formation of *N*-nitroso compounds occurs (a) by acid-catalyzed and bacterial nitrosation in the stomach and (b) via nitric oxide formation during inflammation. The nitrosation reaction is inhibited by ascorbic acid (Tannenbaum *et al.*, 1991), which suggests that endogenous formation of *N*-nitroso compounds is diminished when individuals consume fruits and vegetables (source of ascorbic acid) or ingest vitamin C.

Several similarities and differences between the two classes of N-nitroso compounds are important in consideration of their carcinogenicity. First, nitrosamines require metabolic activation, whereas nitrosamides do not. Second, nitrosamides are less stable due to spontaneous hydrolysis. Third, due to this difference in stability, nitrosamides often act at the site of administration, while nitrosamines act mostly at remote sites. Finally, both nitrosamides and nitrosamines yield similar alkylating intermediates that produce protein and DNA damage.

The 2006 IARC (International Association for Research on Cancer) Working Group in its overall evaluation states that "Ingested nitrate and nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans" (IARC,.2006). Many individual nitrosamines are classified as probable human carcinogens (Group 2A) on the basis of animal studies. The strongest evidence linking N-nitroso compounds to cancer comes from studies of chemotherapy patients. In cancer patients, adjuvant treatment with 1-(2-

chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU) was associated with an increased risk of acute nonlymphocytic leukemia, with a strong dose-response relationship (Boice et al., 1983; 1986). This association provided the basis for the IARC classification of methyl-CCNU as a human carcinogen (class I). In the central nervous system, only nitrosamides (e.g., methylnitrosourea and ethylnitrosourea), and not nitrosamines, were found to be carcinogenic (Lantos,.1986; Lijinsky et al., 1989; Lijinsky,.1999). If a pregnant rat receives a single injection of N-ethyl-N-nitrosourea (ENU), a potent neurocarcinogen, the adult offspring invariably develop glioma, although ENU is cleared from the body of a pregnant animal within minutes (Druckrey et al., 1966; Lantos, 1986; Lijinsky. et al., 1989).

Neither environmental epidemiological studies (on nitrate and nitrite levels in drinking water) (De Roos *et al.*, 2003; Ward et al., 2003; 2006; Zeegers et al., 2006) nor occupational studies (on *N*-nitrosamines in rubber industry) (Straif et al., 2000; Oury et al., 1997) found associations with the majority of cancer types, although positive associations were shown with cancers of the esophagus, oral cavity, and pharynx (Straif. et al., 2000). Straif et al. (2000) also determined a positive association with CNS cancer in rubber industry workers who were exposed for years to various levels of nitrosamines. These investigators calculated that the relative risk (RR) of CNS cancer for these workers was 3.9 (95% CI, 0.3–42.6) with medium exposures to nitrosamines and 6.0 (95% CI, 0.6–57.6) with high exposures after 1 to 10 years of exposure. After 10 or more years of exposure, the RR with medium exposures increased to 5.1 (95% CI, 0.6–45.6) and the RR with high exposures decreased to 3.5 (95% CI, 0.2–56.2). However, these estimates are extremely unstable and therefore, preclude any conclusion on whether non-dietary exposures to N-nitroso compounds are associated with increased risk of brain tumors.

The majority of epidemiological studies focused on dietary N-nitroso compounds. An extensive review of evidence for the role of N-nitroso compounds in the etiology of childhood brain tumors was carried out by Dietrich et al. (2005). The majority of case-control studies (8 out of 10) found a positive association between maternal intake of cured meat (a proxy for exposure to nitrosamides) and childhood brain tumors: the odds ratios (OR) for the highest levels of exposure ranged from 1.1 to 6.04. Less evidence exists for an association with children's cured meat intake (OR ranged from 0.7 to 26.8). Not surprisingly, there is no evidence for a positive association with nitrate from vegetables in the mother's diet, because vegetables are an important source of ascorbic acid, which inhibits endogenous formation of N-nitroso compounds. Confirming the modifying effect of vitamins, a lower risk of childhood brain tumors associated with N-nitroso compounds in maternal diet was seen among those whose mothers took vitamins (Preston-Martin *et al.*, 1996). Studies of adult glioma also provide evidence for neurocarcinogenicity of dietary N-nitroso compounds. A meta-analysis of 9 observational studies indicated that consumption of bacon (RR = 1.31; 95% CI: 1.00–1.71) and ham (RR = 1.64; 95% CI: 1.27–2.14) was associated with increased risk of adult glioma (Huncharek *et al.*, 2003). However, if all studies adjusted for total energy intake, this could attenuate the RR estimates (Huncharek. et al., 2003).

Several types of biomarkers of exposure to *N*-nitroso compounds were used in animal and human studies: urinary metabolites, DNA adducts, and blood protein adducts. Urinary metabolites include *N*-nitrosoproline and 4-(methylnitroso)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Gluc). Persisting for 12–24 hr, urinary biomarkers are useful for short-term exposure assessment. For example, N-nitrosoproline is used as a measure of endogenous nitrosation (Ohshima and Bartsch, 1981; Mirvish,.1995). Both DNA adducts and Hb adducts may be detected two to four months postexposure to N-nitroso compounds in animals and humans and therefore may be used as integrated indices over a period of several months (Hecht,.1998). Biomarkers (urinary, DNA adducts, etc.) for tobacco-specific

nitrosamines were used extensively to monitor the effects of tobacco smoking (Hecht,1998; 2002). For example, 4-(methylnitroso)-1-(3-pyridyl)-1-butanone (NNK) is a systemic lung carcinogen found in tobacco smoke. NNAL and NNAL-Gluc are specific urinary metabolites of NNK in both rats and humans. These biomarkers are higher in subjects exposed to tobacco smoke as compared to controls. Although many urinary biomarkers are transitory in nature, chronic tobacco use produces a consistent measurement of biomarker concentrations (Hecht, 2002). In contrast, levels of HPB (4-hydroxy-1-[3-pyridyl]-1-butanone)-releasing Hb adducts of NNK and N′-nitrosonornicotine (NNN) are low compared to Hb adducts of several other carcinogens and thus may be of limited use as biomarkers in smokers. With respect to dietary exposure, the ethylation and methylation of NH2-terminal valine in Hb were been evaluated as biomarkers of alkylnitrosourea formation from ethylnitrosoureas and methylnitrosoureas, respectively. However, no association was found with intake of cured meats (Gurney *et al.*, 2002). Thus, although multiple biomarkers of exposure to N-nitroso compounds exist, only short-term biomarkers, those persisting for 12–24 hr (urinary metabolites) (Ohshima and Bartsch, 1981) or 2–4 months (DNA and Hb adducts), may be used to assess human exposure to these chemicals.

Polycyclic aromatic hydrocarbons

PAH are a group of over 100 different chemicals that are formed during incomplete combustion of coal, oil and gas, garbage, or other organic substances like tobacco or charbroiled meat (Boffetta et al., 1997; Turusov,1983). PAH are found in at least 600 of 1430 National Priorities List sites identified by the U.S. Environmental Protection Agency (EPA), as evidence of widespread PAH contamination across the United States. PAH are metabolized in the liver and other tissues by the cytochrome P450 enzyme system into reactive electrophiles that bind to cellular macromolecules, including DNA. Resultant DNA adducts, if not repaired, induce mutations that may be involved in carcinogenesis. PAH may also produce cancer indirectly through formation of reactive oxygen species (ROS) (Burdick et al., 2003; Flowers et al., 1996; Rybicki et al., 2006; Shen et al., 2006; Yu et al., 2002).

The earliest evidence of a link between PAH exposure and cancer was suggested by Sir Percival Pott (1714–1788) in his description of squamous cell carcinoma in the scrotum among London men cleaning soot and other residue from chimneys and fireplaces (NTP, 2007; Rao et al., 2008). Pott also hypothesized that a similar cancer on the hands of gardeners, who spread the residue from burning coal as fertilizer, was an occupational disease, eventually determined to be induced by coal tar in the residue. Since that time, many different PAH were shown to induce tumors in the organs of animals, including lung, liver, forestomach, esophagus, mammary gland, and skin (Ross et al., 1995; Cavalieri et al., 1991; Wang et al., 1993; Hayashi et al., 2001; Culp et al., 1998). Further evidence for mutagenicity and carcinogenicity comes from DNA strand breaks, CA, SCEs, MN formation, and mutations of target genes such as HPRT (Gyorffy *et al.*, 2008).

Epidemiological results provide a link between PAH as a class of compounds, but not the specific compounds, and cancers of the lung, oral cavity, larynx, skin, stomach, rectum, cervix, breast, and prostate (Brody et al., 2007; Besaratinia et al., 2002; Rybicki. et al., 2006). Drawing conclusions from occupational studies, IARC classified occupational exposures to PAH in a number of industries as carcinogenic to humans, mainly due to an increased risk of lung, laryngeal, skin, kidney, and urinary bladder cancer (IARC,.1992). In brain tumor epidemiology, only two studies have directly addressed exposure to PAH. Cordier et al. (2004) found that paternal preconceptional exposure to PAH from either tobacco smoke or occupational exposure was associated with an increased risk for childhood brain tumors (OR: 1.4 [95% CI, 1.1–1.7]). No effect was observed for maternal smoking or occupation before conception or during pregnancy (OR: 1.2 [95% CI, 0.6–2.5]). A previous study by the same group found that overall risk of brain tumors, especially astroglial tumors,

was higher for fathers exposed to PAH (OR: 1.2 [95% CI, 0.8–1.9] for astroglial tumors; 2.0 [95% CI, 1.0–4.0] for primitive neuroectodermal tumors; 0.9 [95% CI, 0.4–1.8] for other glial tumors). However, no dose-response relationship was found (Cordier *et al.*, 1997).

Biomarkers of exposure to PAH include urinary metabolites, DNA adducts, and protein adducts (Farmer et al., 2003; Gyorffy. et al., 2008). Urinary 1-hydroxy-pyrene, a biomarker for pyrene, and other metabolites such as benzidine and cotinine was used to assess PAH exposures. Another approach is analysis for a series of 40–50 PAH exposure metabolites in human biological samples (e.g., serum, urine) instead of a single compound. Recent advances in this area include monitoring for derivatives of known carcinogenic compounds (e.g., metabolites of benzo[*a*]pyrene) (Cerna et al., 1997; Myers. et al., 1996). A number of methods exist for measuring PAH-DNA adducts, including high-performance liquid chromatography to specifically measure B[a]p-tetrol, ^{32}P-postlabelling for measuring aromatic DNA adducts including PAH, and immunological methods (ELISA and immunohistochemistry). Elevated levels of PAH-DNA adducts were found in (1) populations exposed to environmental pollution (Farmer. et al., 2003), (2) tissues and amniotic fluid of smokers compared with nonsmokers, (3) various tumor tissues compared with non-tumor tissue (Besaratinia. et al., 2002; Gyorffy. et al., 2008), and (4) maternal and fetal blood. Levels of DNA adducts have correlated with those of protein adducts, including Hb and albumin, in some studies but not in others (Gyorffy. et al., 2008). In addition, several studies found that germ line mutations and polymorphisms in DNA damage response genes are associated with susceptibility to various cancers (Besaratinia. et al., 2002). Future use of these genetic markers may identify those persons who are most at risk from exposure to PAH.

Conclusions

In brain tumor epidemiology, as in other rare cancers, case-control studies are largely the only choice of study design. Currently, epidemiology has not provided a definitive answer for whether occupational and/or environmental exposure to neurocarcinogens increases the risk of brain tumor development. Previous studies suffer from various deficiencies: in occupational cohort studies, brain tumors were not studied as a specific outcome due to their rarity; moreover, the RR estimates from some occupational studies must be interpreted with caution due to potential conflicts of interest (Huff,.2007). However, the most common deficiency in occupational case-control studies is inadequate exposure assessment.

Exposures to environmental carcinogens may be assessed by questionnaire, or by using existing records, such as job title or documents describing concentrations of certain chemicals in the air and/or water at a workplace and/or in certain geographical regions. However, because questionnaires are imprecise exposure assessment tools, the power to detect small increases in the risk of brain tumors associated with a chemical exposure is limited. For example, a food frequency questionnaire as a tool for assessment of dietary AA exposure is unlikely to include all sources of AA exposure; furthermore, even the foods included could have very different levels of AA depending on the manufacturer, source, and the method of preparation. Thus, development of biomarkers is important for further studies in human populations and for pharmacokinetic studies in animals from which human effects may be extrapolated.

Biomarkers of exposure or effect have important advantages over exposure assessment conducted via a questionnaire. These are objective measures that are not liable to be influenced by inaccuracy of recall and, more importantly, to recall bias arising from differences in reporting of suspected harmful exposures by cases and controls. However, most biomarkers reflect recent exposures. Theoretically, the optimal biomarker

measurements involve the levels in the tissues of the target organ of interest. Depending on the cell turnover rates, the levels of such biomarker may be more indicative of long-term exposure. Needless to say that measurements of biomarkers in brain tissues are not applicable to a large-scale epidemiological study, although small validating studies can be conducted using autopsy tissues. Also, the systemic levels of biomarkers measured in circulation or in urine may or may not correlate well with the levels of that same biomarker in the target organ, particularly, if the organ site of interest has different levels of key metabolic and/or DNA repair enzymes expressed. As shown in Table 1, several animal neurocarcinogens can be assessed in humans by using biomarkers from easily accessible biological specimens, such as blood or urine. Urinary biomarkers usually have a very short half-life (hours to days). DNA and Hb adducts, on the other hand, represent an integrated index of exposure over the previous 3–5 months. If the source of the exposure is a lifestyle activity (e.g., driving, smoking, living in urban areas, hobbies) or diet, then it is likely that such exposure represents a steady-state level and would remain fairly constant over a number of years. However, before introducing any biomarker to epidemiological studies, this assumption needs to be tested by comparing questionnaire data with the levels of the DNA and/or Hb adducts.

Epidemiological studies should include both the questionnaire and biomarker assessment. The major role of the biomarkers would be internal validation of the questionnaire-based data (correlation between reported data and biomarker measurements). Such validation should be performed using controls, because in patients diagnosed with cancer, the disease itself, treatment, and/or changes in life-style of may influence the levels of the biomarkers in biological fluids. This validation will result in calculation of attenuation coefficient and as such could be applied to the data analysis of the association between the exposure data and case-control status. With the accumulation of such data, one can begin to postulate the role of animal neurocarcinogens in induction of brain tumors in humans and delineate and prioritize those chemicals for which one needs to obtain a more in-depth evaluation in regard to neurocarcinogenicity and biological exposure information. Finally, it is of note that although the objective of the Second Lebow Conference was to further the field of brain tumor epidemiology, the discussed chemicals are carcinogenic in other organs, and this discussion can be applied to other cancer types.

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Abbreviations

AA	acrylamide
AAMA	acrylamide mercapturic acid conjugate
ENU	*N*-ethyl-*N*-nitrosourea
EPA	U.S. Environmental Protection Agency
GA	glycidamide
GAMA	glycidamide mercapturic acid conjugate
Gluc	glucuronide

HB-val	*N*-(2-hydroxy-3-butenyl)valine
Hb	hemoglobin
pyr	pyrrolidine
HPB	4-hydroxy-1-(3-pyridyl)-1-butanone
IARC	International Agency for Research on Cancer
methyl-CCNU	1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea
NNAL	*N*-nitrosoproline and 4-(methylnitroso)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitroso)-1-(3-pyridyl)-1-butanone
NNN	*N*′-nitrosonornicotine
NTP	National Toxicology Program
OR	odds ratio
PAH	polycyclic aromatic hydrocarbon
pyr-val	N,N-(2,3-dihydroxy-1,4-butadiyl)-valine
RR	relative risk
SCE	sister chromatid exchange
THB-val	N-(2,3,4-trihydroxybutyl)valine

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Table 1

Biomarkers of exposure applicable to human studies

	Biomarkers by Biological Source				
Chemical	**Blood**	**Urine**	**Buccal Cells**	**Nails**	**Breath Condensate**
N-Nitroso compounds	Methylation and pyridyloxobutylation DNA adducts, Hb adducts	*N*-Nitrosoproline, NNAL, NNAL gluc		NNAL	Tobacco-specific nitrosamines
PAHs	PAH-DNA adducts, albumin adducts, Hb adducts	PAH metabolites e.g., 1-hydroxypyrene, 1-hydroxypyrene gluc, 1-naphthol, 2-naphthol	PAH-DNA adducts		
1,3-Butadiene	DNA adducts, Hb adducts (THB-val, HB-val, pyr-val)	mercapturic acids			1,3-Butadiene
Acrylamide	DNA adducts (AA, GA), Hb adducts (AA, GA)	AA, GA, mercapturic acid conjugates (AAMA and GAMA)			

Abbreviations: AA, acrylamide; GA, glycidamide; gluc, glucuronide; Hb, hemoglobin; MA, mercapturic acid; NNAL, *N*-nitrosoproline and 4-(methylnitroso)-1-(3-pyridyl)-1-butanol; PAH, polycyclic aromatic hydrocarbon; pyr, pyrrolidine; THB-val, N-(2,3,4-trihydroxybutyl)valine; val, valine.