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The Relative Toxicity of Compounds in Mainstream Cigarette Smoke Condensate

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Abstract

Many different *in vivo* and *in vitro* tests are currently used to assess the toxicity of chemicals and complex mixtures like cigarette smoke condensate. *In vivo* tests include assays in rodents to determine carcinogenicity, tumorogenicity and reproductive effects. *In vitro* tests of mutagenicity are conducted with both bacterial and mammalian cell systems. A first step toward lowering the toxicity of cigarette smoke condensate is the identification of the relevant compounds. However, changing the concentration of a given smoke component may not linearly alter the biological activity of the complex mixture due to interactive effects. The "effective toxicity" of a chemical constituent is a function of the concentration, the metabolic fate, the potency in *in vivo* and *in vitro* assays, and the ability to reach the target tissues. The logarithm of the octanol-water partition coefficient (log P) is an important parameter as it affects metabolism, biological transport properties and intrinsic toxicity. Using concentration data from the International Agency for Cancer Research (IARC), biological activity data from the Registry of Toxic Effects of Chemical Substances (RTECS) database and measured and calculated log P values, we have rank ordered some of the important compounds in cigarette smoke condensate by their measured or potential toxicity. Compounds of particular concern may include hydroquinone, catechol, 4-methylcatechol, 4-vinylphenol and 4-vinylguaiacol, based on their relatively high concentration and negative σ^+ values. Condensates from different cigarette brands, tar categories and styles vary in their concentrations of these compounds. Chemicals of greater commercial or scientific interest may be toxicity tested more extensively, thereby increasing the probability of positive test results and highlighting the need for consideration of structure-activity relationships.

Introduction

Previously, we published an international literature survey of the nine IARC Group I carcinogens reported in mainstream cigarette smoke (Smith *et al.*, 1997). These nine compounds are benzene, cadmium, arsenic, nickel, chromium, 2-naphthylamine, vinyl chloride, 4-aminobiphenyl and beryllium. Other researchers also consider polonium-210 and ethylene oxide to be members of this list (Hoffmann, 1998). In this review, several methods were recommended toward minimizing the concentration of these compounds in mainstream smoke. These methods included using fertilizers low in nitrogen and heavy metals and employment of cigarette design features such as enhanced charcoal filtration and primarily heating rather than burning tobacco. The purpose of the current study is to broaden the effort to reduce the toxicity of mainstream cigarette smoke by first developing a rational scheme for ranking the organic compounds in mainstream tobacco smoke by their "effective toxicity."

Several factors may contribute to the "effective toxicity" of a mainstream smoke condensate constituent including the concentration, metabolic fate, potency of measured biological activities and lipophilicity of the constituent. Knowledge regarding the potential "effective toxicity" of tobacco smoke condensate constituents may provide targets for selective removal.

At least four thousand compounds have been identified in cigarette smoke (Dube and Green, 1982). Condensates of this complex mixture are toxic in several *in vitro* tests including: bacterial mutagenesis (Ames *Salmonella* assay); chromosomal aberrations in Chinese hamster ovary (CHO) cells; sister chromatid exchanges in CHO cells; and unscheduled DNA synthesis in rat hepatocytes (Doolittle *et al.*, 1990; Lee *et al.*, 1990). In addition, cigarette smoke condensate (CSC) repetitively painted on the skin of rodents has produced benign and malignant tumors

(Hoffmann *et al.*, 1983). However, direct extrapolations of biological activity results between CSC and cigarette smoke exposures are inappropriate because of differences in chemical composition and physicochemical properties.

Lipophilicity, as measured by the base 10 logarithm of the octanol-water partition coefficient (P) and denoted as log P, is included as a contributory factor to the "effective toxicity." Log P correlates with a number of biological activites including *in vitro* mutagenicity and carcinogenicity in rodents (Debnath *et al.*, 1994). Lipophilic compounds can cross biological barriers which contain lipid, e.g., cell or microsomal membranes and skin (Tayar *et al.*, 1991). Therefore, log P influences metabolic fate, intrinsic biological activity and the biological transport properties of chemicals (Hansch and Leo, 1995a).

The relationship between log P and toxicity is sometimes not straightforward. Since the kidneys can excrete hydrophilic compounds more readily than hydrophobic compounds (Rozman and Klaassen, 1996), several metabolic pathways in the liver seek to convert hydrophobic compounds into hydrophilic species (Parkinson, 1996). Occasionally, this attempted detoxification process instead leads to the formation of reactive electrophilic metabolites which can bind DNA and proteins (Hoffmann GR, 1996). Therefore, many hydrophobic compounds are inadvertently converted to chemical species with increased toxicity. This process is facilitated by high log P values. Hydrophobicity also favors the induction of cytochrome P450 enzymes (Hansch and Leo, 1995a).

The relationship between log P and toxicity is further complicated by the association of compounds having log P greater than 2.0 with increased residence time in lipid-containing structures (Hansch *et al.*, 1987). Thus, compounds with very high log P values, e.g., the pesticide dichlorodiphenyltrichloroethane (DDT) with a log P of 6.91, can bioaccumulate and

cause long-term adverse effects (Hansch and Leo, 1995a). Additionally, hydrophobic compounds in sufficient concentration can display nonspecific toxicity by perturbing the protective lipid membrane bilayer surrounding cells (Hansch and Leo, 1995b). Therefore, while log P value is a predictor of toxicity, the range of log P values associated with optimum toxicity is influenced both by the chemical structure of the compound and by the particular type of toxicity.

Methods

Selection of Compounds for Ranking

The 122 compounds selected for ranking were taken from "Table 19 - Concentrations of biologically active agents in non-filter cigarette mainstream smoke" and "Appendix 2 - Chemical compounds identified in tobacco smoke that have been evaluated for carcinogenicity in the *IARC Monographs* series" (IARC, 1986).

Categorization of Potency of Biological Activity

Results of *in vitro* and *in vivo* biological activity tests on the 122 compounds are compiled in the Registry of Toxic Effects of Chemical Substances (RTECS) database. RTECS is a comprehensive database containing toxicity information for over 10,000 chemicals. These compiled results were used to develop a categorization scheme consisting of six different categories of biological activity (Table I). Category 1, rodent carcinogen + reproductive effector, is the most potent category. Category 6, insufficient evidence of biological activity, is the least potent category. When a compound fulfilled the criteria for more than one category, it was assigned the higher category number. Each of the 122 compounds was assigned a biological activity category number.

A reproductive effector is a compound that adversely altered birth-weight or litter size, or produced a teratogenic effect in some animal species, usually a rodent. The rationale underlying the categorization scheme is that a compound which both produces cancer and adverse reproductive effects (Category 1) is of greater concern than a compound which only produces cancer (Category 2). Results from feeding studies in monkeys, rats and mice, in which the potent mutagenic heterocyclic amines were tested for their carcinogenic potential and displayed large species effects (Adamson *et al.*, 1996), argue against overemphasizing quantitative potency differences between rodent carcinogens within Category 1 and 2.

Reproductive effectors (Category 3) are classified as more biologically active than benign tumorigens (Category 4) because of the possibility that even reproductive effectors not reported to be teratogens might possess some teratogenic potential. In contrast, since most rodent bioassays are conducted at or near the maximum tolerated dose (MTD) (Ames and Gold, 1991), benign tumorigens were believed not to possess significant carcinogenic potential, although the possibility certainly exists. Compounds previously shown to be mutagenic in an *in vitro* test system (Category 5) are considered more significant than Category 6 compounds not yet shown to possess adverse biological activities.

Determination of Octanol-Water Partition Coefficients

The logarithm of the octanol-water partition coefficient was either measured or calculated for each of the 122 compounds (Leo, 1993). Experimental octanol-water log P values were determined by the shake-flask method (Hansch and Leo, 1995c).

Calculation of Effective Average Concentration

The Effective Average Concentration is calculated as the product of the "average concentration," expressed in micrograms delivered per cigarette, times the percent of the total

concentration partitioning into the octanol phase. Thus, the Effective Average Concentration is the number of micrograms of the compound delivered per cigarette which would be found in the lipid phase of a compartmentalized system. Due to the higher toxicity of hydrophobic compounds as compared with hydrophilic compounds, this calculated product term more accurately reflects the potential contribution to toxicity than does "average concentration."

Collection of Cigarette Smoke Condensate

Mainstream cigarette smoke is a complex aerosol consisting of a vapor phase and a particulate phase. Some chemical constituents, for example carbon monoxide and carbon dioxide, are found primarily in the vapor phase while others, such as nicotine, predominate in the particulate phase. Some compounds, such as phenol, are found in both the vapor and particulate phases (Guerin *et al.*, 1992). Several different methods are commonly used to collect and measure the amount of a chemical compound in mainstream cigarette smoke. Gravometric methods using Cambridge filters are used for the collection of particulate matter (Pillsbury *et al.*, 1969). Electrostatic precipitation is another method of collecting particulate matter from whole cigarette smoke (Coresta, 1968). In contrast, 'cold trap' methods collect compounds from the vapor phase as well as the particulate phase (Dube and Green, 1982).

Results

Table II lists the 122 compounds sorted within the six biological activity categories by ascending order of Effective Average Concentration. Log P and P values are given for each compound.

Each of the six categories contains groups of compounds that are relatively hydrophobic. The average log P values range from a low of 1.72 ± 2.17 for Category 1 compounds to a high of

4.55 ± 2.11 for Category 4 compounds. A log P of 1.72 for a Category 1 compound means that the compound is 52.5 times more soluble in octanol than in water, while the high value of log P = 4.55 means that a Category 4 compound is 35,481 times more soluble in octanol. The high lipophilicity of these compounds is not surprising because the original IARC list of 122 compounds was selected on the basis of biological activity and high lipophilicity correlates strongly with biological activity. This pre-selection process precludes correlation of log P values with biological potency category scores.

A preliminary analysis of Table II reveals at least 14 compounds of particular interest based on Effective Average Concentration values and Toxicity Category. Within the most potent Category 1 (Rodent Carcinogen + Reproductive Effector), significant lipid-soluble amounts per cigarette of acetaldehyde (427 μg), phenol (97 μg), hydroquinone (96.7 μg), formaldehyde (37 μg) and benzene (30 μg) are found. Three of the five Category 1 compounds listed above are considered by IARC to possess carcinogenic risk in humans (IARC, August 27, 1997). IARC classifies benzene as a Group I compound known to cause cancer in humans. Similarly, formaldehyde is placed in Group 2A (probable human carcinogen) and acetaldehyde into Group 2B (possible human carcinogen) by IARC. In contrast, hydroquinone and phenol are considered by IARC to be "unclassifiable" as to their carcinogenic risk in humans.

The conservative nature of the databases herein employed over-classifies phenol. Several laboratories have reported that phenol is a weak tumor promoter (Salaman and Glendenning, 1957; Boutwell and Bosch, 1959; Van Duuren *et al.*, 1968). For example, in the Boutwell and Bosch (1959) report, the production of papillomas and carcinomas on the skin of mice treated with phenol alone was observed. The dose of phenol administered was sufficiently high so that during the first six weeks of treatment many of the mice bore wounds and showed

reparative hyperplasia. These authors also reported that "papillomas appeared rapidly and in large numbers after treatment with dimethylbenzanthracene (DMBA) followed by repeated applications of a 10 percent solution of phenol," while carcinomas appeared more slowly. In contrast, administration of lower doses of phenol has not induced tumors. In fact, the skin carcinogenicity in mice of an initiating dose of 150 µg benzo[a]pyrene (B[a]P) was reduced by approximately 50% by three times weekly administration of 3 mg of phenol (Van Duuren and Goldschmidt, 1976). Negative carcinogenicity results have also been reported by the National Toxicology Program of the National Cancer Institute (Carcinogenesis Testing Program, 1980). In this experiment, mice were exposed for 103 weeks to drinking water containing either 2,500 or 5,000 ppm phenol. Similarly, a small, short-term feeding study in rats failed to induce forestomach lesions after administering 2% phenol (Altmann *et al.*, 1986). On balance, phenol should not be considered a rodent carcinogen.

Results regarding the developmental or teratogenic potential of phenol are similarly equivocal, but probably negative. In the absence of maternal toxicity, phenol reportedly reduced fetal weights in rats, but was not teratogenic (Price *et al.*, 1986). Similarly, Minor and Becker (1971) did not observe phenol teratogenicity in rats. Also in rats, Kavlock (1990) did observe kinked tails after high-dose acute prenatal exposure to phenol. In a later experiment, Narotsky and Kavlock (1995) also observed kinked tails in one litter of phenol-exposed rats in the presence of maternal toxicity. In the presence of maternal toxicity in mice, phenol caused growth retardation, abnormal structural development, cleft palate and prenatal mortality (Price *et al.*, 1986). Overall, phenol appears to be a "reproductive effector," as defined by low birth weights, but not a teratogen at levels below maternal toxicity. Therefore, phenol is better classified as a Category 3 compound, i.e., a reproductive effector.

Similarly, the categorization of acetaldehyde also requires further discussion. A number of *in vitro* studies have been conducted which show adverse interactions between acetaldehyde and DNA. At relatively nontoxic acetaldehyde concentrations, DNA adducts were formed in a dose-dependent manner in cultured human buccal epithelial cells (Vaca *et al.*, 1998). Single-strand breaks in human lymphocyte DNA have been reported at a concentration of 1.56 mM acetaldehyde (Singh and Khan, 1995). In a study employing "shuttle vector plasmids" replicated in human cells, Matsuda *et al.* (1998) have reported that acetaldehyde induces GG to TT base substitutions. Despite its *in vitro* mutagenicity, clastogenicity (Ristow *et al.*, 1995) and cytotoxicity (Seitz and Poschl, 1997), inspired acetaldehyde in the rat is carcinogenic only at concentrations sufficient to overwhelm nasal aldehyde dehydrogenase detoxification capacity (Morris, 1997). Additionally, acetaldehyde induces a wide range of congenital abnormalities if administered to pregnant animals during embryogenesis or early organogenesis (Kaufman, 1997). The teratogenic mechanism is believed to involve cytoskeletal disruption leading to neural and cardiac defects (O'Shea and Kaufman, 1981). Therefore, the categorization of this compound is problematic, with a conservative view possibly considering acetaldehyde to be both a "rodent carcinogen" and a "reproductive effector," i.e., a Category 1 compound. Alternatively, a classification of Category 3 as a "reproductive effector" and a "mutagen" may be more appropriate.

In Category 2 (Rodent Carcinogens), 36 lipid-soluble micrograms per cigarette of 4-methylcatechol are reported. This compound is "not classified" by IARC for carcinogenic risk in humans. Several of the Category 3 (Reproductive Effector) compounds are also found in significant amounts in mainstream cigarette smoke condensate. These "reproductive effectors" include the following lipid-soluble amounts: acetic acid (388 µg); catechol (172 µg); acetone

(102 μg); methanol (23 μg); acrolein (80.9 μg); 3-cresol (59 μg); 4-vinylphenol (31.1 μg); and 4-vinylguaiacol (11 μg).

Discussion

In Table II, it is interesting that several of the most toxic compounds are phenols and that benzene may be metabolically converted via cytochrome P450 to phenol. These toxic phenolic compounds include hydroquinone (Category 1); 4-methylcatechol and 2,4-dimethylphenol (Category 2); phenol, catechol, 3-cresol, 4-vinylphenol and 4-vinylguaiacol (Category 3). Some perspective on the biological activity of phenols may be provided by the discovery that the toxicity of phenols to rat embryos appears to be related to their ability to form free radicals (Hansch *et al.*, 1995). Exposing rat embryos to simple phenols resulted in various forms of maldevelopment. This maldevelopment could be correlated with the Hammett parameter Sigma plus, σ^+ , for substituted phenols as seen in the following quantitative structure-activity relationship (QSAR) regression Equation 1 (Hansch *et al.*, 1995):

$$\log 1/C = -\rho\sigma^+ + \text{constant} \quad \text{(Equation 1)}$$

where C is the molar concentration of phenol producing a "standard" deformation and ρ is the reaction constant. Although the quality of the correlation was not high ($r^2 \approx 0.83$), four different types of endpoints showed the same dependence of ρ on σ^+. A review of the literature (Hansch and Gao, 1997) revealed that in 25 QSAR equations describing the formation of free radicals by abstracting H· from the phenolic hydroxy group, 23 equations were correlated with σ^+.

These results on free radical formation sparked an *in vitro* study of the toxicity of phenols to L1210 leukemia cells. This cancer cell line was selected because its rapidly dividing cells (similar to the embryo situation) generate increased amounts of reactive oxygen, thereby hypothetically increasing the potential for teratogenic and mutagenic activity. An examination

of the growth inhibition patterns of 37 phenols with substituents at the 3- or 4- position was conducted. From this L1210 leukemia study, the following QSAR was formulated for the 23 electron releasing substituents in substituted phenols (Selassie *et al.*, 1998):

$$\log 1/IC_{50} = -1.58\sigma^{+} + 0.21\log P + 3.10 \quad n = 23,\ r^2 = 0.898,\ s = 0.191 \text{ (Equation 2)}$$

In Equation 2, IC_{50} is the molar concentration of phenol causing 50% growth inhibition of L1210 leukemia cells in 48 hours. Toxicity depends on the ability of the phenol substituent to increase electron density in the aromatic ring, thereby facilitating H· abstraction.

The toxicity of substituted phenols has been correlated with σ^{+} values. This observation implies that following H· abstraction from the phenol hydroxy group, the remaining phenoxy radical has some electron-deficient character. Such a species would be stabilized by electron-donating substituents such as amino, hydroxy, methoxy and methyl groups and presumably would possess an increased lifetime to facilitate biological/cellular damage. Substituted phenols bearing electron-donating substituents would be predicted to be more toxic.

Toxicity also depends on hydrophobicity. Although the coefficient of the hydrophobic term in Equation 2 is relatively small, i.e., 0.21, it is nonetheless important. For example, the log P for 4-aminophenol is 0.04 and for the environmental estrogen nonylphenol it is 6.21. Hence the value for the coefficient 0.21, times the log P of 6.21, is ≅ 1.3. The comparable coefficient times log P product for 4-aminophenol is 0.21 x 0.04 = 0.008. Taking the anti-log of each product to find the contribution of hydrophobicity, gives a 10^{x} value of 19.95 for nonylphenol and a 10^{x} value of 1.02 for 4-aminophenol. Therefore, the contribution of hydrophobicity to nonylphenol toxicity is about 20 times that for 4-aminophenol. Not only is nonylphenol well fit by this equation, other estrogens including octylphenol, bisphenol A, diethylstilbesterol and estradiol also fit this QSAR equation. Of course, phenol and hydroquinone fit the expression.

In Equation 2, the negative dependence on σ^+ strongly resembles what is observed in the developmental toxicity of phenols on rat embryos, as well as for the radical abstraction of a hydrogen atom from phenolic groups. The other 14 electron-withdrawing substituted phenols (37 total – 23 with electron releasing substituents) clearly show a linear dependence on hydrophobicity alone, as seen in Equation 3:

$$\log 1/IC_{50} = 0.62 \log P + 2.35 \quad \text{(Equation 3)}$$

The dichotomy in mechanism of action of this large set of diverse phenols is unique. It suggests that two distinct processes are operative. In the case of electron-donating substituted phenols, the observations are consistent with a radical-mediated process. In contrast, with electron-withdrawing substituted phenols, non-specific toxicity as modulated by hydrophobicity, appears to predominate.

As described earlier in the Results section, phenol is interesting in that it does not appear to be carcinogenic. As the baseline phenolic compound, it is well predicted by either Equation 2 or 3. As discussed above, the evidence for its carcinogenicity is weak, but the small change of adding a para-methoxy moiety to phenol yields a carcinogenic compound (Asakawa *et al.*, 1994). This is to be explained by the σ^+ values of 0 for phenol and -0.78 for methoxyphenol. The carcinogenic potential of many phenols may be estimated from Equation 2 or 3.

With methyl substituents having negative σ^+ values similar to the previously stated free radical generating QSARs, 4-methylcatechol and 2,4-dimethylphenol fall into the relatively potent Category 2 (rodent carcinogens). Since the toxicity category ranking number may be influenced by the amount of testing, it may not be surprising that catechol and vinylphenol fall into Category 3 and the cresols are only in Category 4, even though their chemical structures would predict higher toxicities.

It is only very recently that phenol toxicity related to σ^+ has been established. Scores of studies on the toxicity of phenols in various biological systems have missed this connection. In many instances, the relationship was missed because the appropriate substituents were not studied. Also, the effects may not be shown by using slow growing cells with inadequate free radical generating ability. In summary, the evidence in hand suggests that phenols in smoke may exhibit their toxic action via phenoxy radicals formed during the combustion process or by oxidation in lung cells.

There have been significant and steady reductions in 'tar' yields since the mid-1950s (Wynder and Hoffmann, 1979). These 'tar' reductions have been achieved by several methods including reduced tobacco weight, improved filtration, air dilution and agronomic practices. These modifications also significantly reduced yields of constituents associated with the vapor phase. Therefore, chemical reductions reported in mainstream cigarette smoke have already been achieved.

Chemists and toxicologists attempting to further reduce the toxicity of mainstream cigarette smoke are faced with a difficult task. The toxicity of individual cigarette smoke components sometimes differs from the toxicity resulting from the same concentration of that component within a complex mixture (Lee *et al.*, 1994; Lee *et al.*, 1996). Although interactive effects are sometimes operant, reductions in chemical concentration and complexity resulting from primarily heating rather than burning tobacco (deBethizy *et al.*, 1990) and also from enhanced charcoal filtration and use of a low nitrogen tobacco blend (Bombick *et al.*, 1997), have demonstrated significant reductions in toxicity. Therefore, removing biologically active compounds from mainstream cigarette smoke is a logical step toward reducing toxicity. The question then becomes, "Which compounds merit the highest priority for removal?"

The usefulness of the scheme in Table II for ranking the toxicity of cigarette smoke condensate constituents is affected by the quality of the data used to develop it. The interpretation of the results from the *in vitro* and *in vivo* studies compiled in the RTECS database is complicated by theoretical and practical considerations. On a practical level, chemicals of greater commercial or scientific interest would tend to have been tested more extensively, thereby increasing the probability of positive test results. This tendency could lead to relatively higher potency category values.

In vitro test results do not necessarily correlate with results from *in vivo* tests. Zeiger (1987) has reviewed the correlation issue for *Salmonella typhimurium* Ames test assays. He analyzed the results for 224 chemicals tested in long-term studies for carcinogenicity in rats and mice by the National Cancer Institute and Ames tested by the National Toxicology Program. Zeiger reported that, "A clear mutagenic or equivocal response in *Salmonella* was predictive for 77% of the carcinogens or equivocal carcinogens, although only 54% of the 149 carcinogens or equivocal carcinogens were mutagens, and 58% of the nonmutagens were carcinogens or equivocal carcinogens. The proportion of mutagens and equivocal mutagens that were not carcinogenic or equivocal was 23%."

In vivo tests conducted at or near the maximum tolerated dose (MTD) have come under criticism (Ames and Gold, 1991). These authors argue that animals exposed to these near toxic doses for long periods of time may develop tumors due to elevated rates of mitogenesis rather than due to any specific genotoxic effect of the chemical being tested. In support of this hypothesis, they note that about half of all chemicals tested chronically at the MTD are carcinogens.

A further challenge inherent in any approach for reducing the toxicity of mainstream cigarette smoke is the prevailing concept of what constitutes a toxic response. When a chemical is subjected to a specific test, e.g., for neurotoxicity or ability to bind to estrogen receptors, the results are relevant only to that particular test or battery of tests. Other assays for carcinogenicity, tumorigenicity or mutagenicity might give contrasting results. Therefore, a comprehensive view of the toxicity of a chemical should include results from any *in vitro* or *in vivo* tests and should also consider toxicity predicted by structure-activity relationships. The dissatisfaction with the current ability to predict the toxicity of complex mixtures emphasizes the importance of increasing understanding of the quantitative structure-activity relationships underlying toxicity.

Determining the toxicity of chemicals from the available literature is not an exact process. The potency of chemicals assayed in similar tests can be compared in a relative way, but correlations to effects in humans are not yet possible. Concentrations of chemicals in mainstream smoke condensate can be directly compared, but the composition of mainstream smoke condensate may differ somewhat from that of mainstream smoke. Additionally, the variability in the concentration of chemical yields from different cigarettes is considerable, e.g., values from 0 to 104 micrograms of benzene per cigarette have been reported (Smith *et al.*, 1997).

Other groups have previously attempted to provide lists of the major toxic agents in cigarette smoke by using an empirical, weight of evidence approach emphasizing cancer related endpoints. Most notably, the American Health Foundation (AHF) in Valhalla, New York, has correlated a large body of their own data and additional literature on compounds of interest in tobacco smoke (Hoffmann, 1998). The categorization scheme used in this study qualitatively

overlaps with the AHF list on some compounds, e.g., phenol, catechol, acrolein, acetaldehyde and benzene. The major difference in the two lists is the greater emphasis placed on the role of N-nitrosamines and polycyclic aromatic hydrocarbons by the AHF (Hoffmann and Hoffmann, 1997).

In summary, based on the 1986 IARC Monograph list as a starting point, the following compounds may represent initial targets for further reducing the biological activity of cigarette smoke: hydroquinone, catechol, 4-methyl catechol, 4-vinylphenol and 4-vinylguaiacol. If the relative concentrations of chemical components from a cigarette other than that employed by IARC had been used as the starting point, this initial target list would somewhat differ. Therefore, this approach leads to a flexible ranking process, and is not intended to provide a rigid list applicable under all conditions. Any system should be periodically updated to include new information from *in vitro* and *in vivo* assays and advances in understanding of structure-activity relationships. While cancer risk will remain a preeminent concern, the EPA's recent decision to test 7,000 chemicals for their potential to disrupt hormone systems in humans and wildlife (Hileman, 1998) illustrates the evolutionary nature of the concept of "toxicity."

Since the first series of large retrospective, case-control studies on lung cancer and cigarette smoking were published in 1950, a number of specific compounds and families of compounds have been proposed as the causative agents (Huber, 1989). None of these hypotheses have become widely accepted due to recognition of potential interactive effects within the complex mixture and shortfalls in mechanistic understanding. Hopefully, this general approach which simultaneously attempts to consider exposure, biological availability (log P), intrinsic electronic reactivity and a variety of measured toxic responses, can more successfully chase the

moving target of evaluating the relative toxicity of individual components within a complex mixture.

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Table I. Categorization Scheme

Category	Description	Abbreviation
1	Rodent Carcinogen + Reproductive Effector	Ca + Re
2	Rodent Carcinogen	Ca
3	Reproductive Effector	Re
4	Benign Tumorigen	Tu
5	*In vitro* Mutagen	Mu
6	Insufficient Evidence of Biological Activity	IE

Table II. Effective Average Concentration and Toxicological Potency [§]

CAS #	Compound Name	Avg. Conc. (µg/cig.)	log P	P	Effec. Avg. Conc. (µg/cig.)	Toxicity Category
75-07-0	Acetaldehyde	709	-0.22[c]	0.60	427	1
108-95-2	Phenol	100	1.46	28.84	97	1
123-31-9	Hydroquinone	121.5	0.59	3.89	96.7	1
50-00-0	Formaldehyde	54	0.35	2.24	37	1
71-43-2	Benzene	30	2.13	134.90	30	1
79-46-9	2-Nitropropane	0.97	0.55[c]	3.55	0.76	1
50-32-8	Benzo[a]pyrene	0.03	5.97	933,254	0.03	1
62-75-9	N-Nitroso-dimethylamine	0.1005	-0.57	0.27	0.0271	1
51-79-6	Urethane	0.029	-0.15	0.71	0.021	1
55-18-5	N-Nitroso-diethylamine	0.01	0.48	3.02	0.01	1
75-01-4	Vinylchloride	0.0085	1.52[c]	33.11	0.0083	1
100-75-4	N-Nitroso-piperidine	0.009	0.63	4.27	0.007	1
224-42-0	Dibenz[a,j]acridine	0.0065	5.76[c]	575,440	0.0065	1
924-16-3	N-Nitroso-di-n-butylamine	0.0015	1.92	83.18	0.0015	1
621-64-7	N-Nitroso-di-n-propylamine	0.001	1.36	22.91	0.001	1
226-36-8	Dibenz[a,h]acridine	0.0001	5.76[c]	575,440	0.0001	1
452-86-8	4-Methylcatechol	38	1.38[c]	23.99	36	2
105-67-9	2,4-Dimethylphenol	1.1	2.30	199.53	1.1	2
1133-64-8	N'-Nitrosoanabasine	0.2	1.12[c]	13.18	0.19	2
16543-55-8	N'-Nitrosonornicotine	0.19	0.57[c]	3.72	0.15	2
64091-91-4	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol	0.39	-0.45[c]	0.35	0.14	2
56-55-3	Benz[a]anthracene	0.055	5.79	616,595	0.055	2
87-62-7	2,6-Dimethylaniline	0.054[a]	1.84	69.18	0.053	2
930-55-2	N-Nitrosopyrrolidine	0.022	-0.19	0.65	0.014	2
205-82-3	Benzo[j]fluoranthene	0.0135	6.12[c]	1,318,257	0.0135	2
205-99-2	Benzo[b]fluoranthene	0.013	6.12[c]	1,318,257	0.013	2
193-39-5	Indeno[1,2,3-cd]pyrene	0.012	6.58[c]	3,801,894	0.012	2
91-59-8	2-Naphthylamine	0.0115	2.28	190.55	0.0114	2

191-26-4	Anthanthrene	0.011	6.58[c]	3,801,894	0.011	2
53-70-3	Dibenz[a,h]anthracene	0.004	6.50	3,162,278	0.004	2
92-67-1	4-Aminobiphenyl	0.0035	2.88	758.58	0.0035	2
10595-95-6	N-Nitrosomethylethylamine	0.00505	-0.24[c]	0.58	0.00290	2
1116-54-7	N-Nitrosodiethanolamine	0.09	-1.58[c]	0.03	0.0027	2
189-55-9	Dibenzo[a,i]pyrene	0.0025	7.30[c]	19,952,623	0.0025	2
194-59-2	7H-Dibenzo[c,g]carbazole	0.0007	5.86[c]	724,436	0.0007	2
3697-24-3	5-Methylchrysene	0.0006	6.16[c]	1,445,439	0.0006	2
189-64-0	Dibenzo[a,h]pyrene	-	7.30[c]	19,952,623	-	2
191-30-0	Dibenzo[a,l]pyrene	-	7.30[c]	19,952,623	-	2
54-11-5	Nicotine	1650	1.17	14.79	1546	3
64-19-7	Acetic acid	570	-0.17	0.68	388	3
120-80-9	Catechol	195	0.88	7.59	172	3
67-64-1	Acetone	175	-0.24	0.58	102	3
107-02-8	Acrolein	82.5	-0.01	0.98	80.9	3
108-39-4	3-Cresol	60	1.96	91.20	59	3
2628-17-3	4-Vinylphenol	31.3	2.20[c]	158.49	31.1	3
67-56-1	Methanol	135	-0.77	0.17	23	3
7786-61-0	4-Vinylguaiacol	11	2.05[c]	112.20	11	3
90-15-3	1-Naphthol	0.27	2.84	691.83	0.27	3
62-53-3	Aniline	0.25	0.90	7.94	0.22	3
95-53-4	o-Toluidine	0.115	1.32	20.89	0.110	3
192-97-2	Benzo[e]pyrene	0.016	6.12[c]	1,318,257	0.016	3
91-64-5	Coumarin	-	1.39	24.55	-	3
64-18-6	Formic acid	340	-0.54	0.29	98.6	4
106-44-5	4-Cresol	60	1.94	87.10	59	4
1706-01-0	3-Methylfluoranthene	40	5.45[c]	281,838	40	4
108-46-3	Resorcinol	44	0.80	6.31	38	4
33543-31-6	2-Methylfluroanthene	30	5.45[c]	281,838	30	4
110-86-1	Pyridine	28	0.65	4.47	23	4
95-48-7	2-Cresol	22	1.95	89.13	22	4
97-53-0	2-Methoxy-4-(2-propenylphenol)	3	2.40[c]	251.19	3	4
71267-22-6	N'-Nitrosoanatabine	1.85	1.12[c]	13.18	1.72	4
86-74-8	Carbazole	1	3.72	5,248	1	4

85-01-8	Phenanthrene	0.345	4.46	28,840	0.345	4
206-44-0	Fluoranthene	0.18	5.16	144,544	0.18	4
120-12-7	Anthracene	0.1265	4.45	28,184	0.1265	4
238-84-6	Benzo[a]fluorene	0.1145	5.68	478,630	0.1145	4
95-68-1	2,4-Dimethylaniline	0.057	1.68	47.86	0.056	4
218-01-9	Chrysene	0.05	5.73	537,032	0.05	4
191-24-2	Benzo[g,h,i]perylene	0.0315	6.63	4,265,795	0.0315	4
832-69-9	1-Methylphenanthrene	0.03	5.03	107,152	0.03	4
243-17-4	Benzo[b]fluorene	0.02	5.77	588,844	0.02	4
217-59-4	Triphenylene	0.02[a]	5.49	309,030	0.02	4
224-41-9	Dibenz[a,j]anthracene	0.01	6.84[c]	6,918,310	0.01	4
207-08-9	Benzo[k]fluoranthene	0.009	6.12[c]	1,318,257	0.009	4
95-78-3	2,5-Dimethylaniline	0.00872	1.83	67.61	0.00860	4
1705-85-7	6-Methylchrysene	0.007	6.16[c]	1,445,440	0.007	4
3351-31-3	3-Methylchrysene	0.006	6.16[c]	1,445,440	0.006	4
198-55-0	Perylene	0.004	5.82	660,693	0.004	4
203-12-3	Benzo[g,h,i]fluoranthrene	0.0025	5.41[c]	257,040	0.0025	4
3351-32-4	2-Methylchrysene	0.001	6.16[c]	1,445,440	0.001	4
191-07-1	Coronene	0.001	7.04[c]	10,964,782	0.001	4
86-73-7	Fluorene	-	4.18	15,136	-	4
195-19-7	Benzo[c]phenanthrene	-	5.66[c]	457,088	-	4
3351-30-2	4-Methylchrysene	-	6.16[c]	1,445,440	-	4
215-58-7	Dibenz[a,c]anthracene	-	6.17	1,479,108	-	4
192-65-4	Dibenzo[a,e]pyrene	-	7.30[c]	19,952,623	-	4
205-12-9	Benzo[c]fluorine	-	5.10[c]	125,893	-	4
123-73-9	Crotonaldehyde	15	0.69[c]	4.90	12	5
90-05-1	2-Methoxyphenol	13	1.32	20.89	12	5
109-06-8	2-Picoline	12.3	1.11	12.88	11.4	5
97-54-1	2-Methoxy-4-propenylphenol	9	2.58[c]	380.19	9	5
108-47-4	2,4-Lutidine	1.7	1.64[c]	43.65	1.7	5
109-08-0	2-Methylpyrazine	2.2	0.23	1.70	1.4	5
108-48-5	2,6-Lutidine	1.4	1.68	47.86	1.4	5
129-00-0	Pyrene	0.125	4.88	75,858	0.125	5
106-49-0	p-Toluidine	0.034	1.39	24.55	0.033	5
87-59-2	2,3-Dimethylaniline	0.027[a]	1.81[c]	64.57	0.027	5

589-16-2	4-Ethylaniline	0.027[a]	1.96	91.20	0.027	5
2243-47-2	3-Aminobiphenyl	0.005	2.80[c]	630.66	0.005	5
134-32-7	1-Naphthylamine	0.0035	2.25	177.83	0.0035	5
90-41-5	2-Aminobiphenyl	0.003	2.84	691.83	0.003	5
6053-02-7	4-Vinlylcatechol	84	1.60[c]	39.81	82	6
488-17-5	3-Methylcatechol	38	1.38[c]	23.99	36	6
124-39-6	4-Ethylcatechol	28	1.91[c]	81.28	28	6
108-99-6	3-Picoline	24.1	1.20	15.85	22.7	6
108-89-4	4-Picoline	24.1	1.22	16.60	22.7	6
1121-55-7	3-Vinylpyridine	22.5	1.37[c]	23.44	21.6	6
620-17-7	3-Ethylphenol	18.5	2.40	251.19	18.5	6
123-07-9	4-Ethylphenol	18.5	2.58	380.19	18.5	6
1300-20-7	Xylenol	7	2.42[c]	263.03	7	6
589-93-5	2,5-Lutidine	3.9	1.64[c]	43.65	3.8	6
695-84-1	2-Vinylphenol	3.8	2.20[c]	158.49	3.8	6
458-35-5	Coniferyl alcohol	1	0.59[c]	3.89	0.8	6
139-19-3	2-Naphthol	0.54	2.70	501.19	0.54	6
5910-89-4	2,3-Dimethylpyrazine	0.4	0.64[c]	4.37	0.3	6
587-02-0	3-Ethylaniline	0.057[a]	1.94[c]	87.10	0.056	6
578-54-1	2-Ethylaniline	0.054[a]	1.74	54.95	0.053	6
1576-67-6	3,6-Dimethylphenanthrene	0.034	5.44[c]	275,423	0.034	6
108-44-1	m-Toluidine	0.03	1.40	25.12	0.03	6
2246-44-8	2-Methyl-1-naphthylamine	0.004	2.54[c]	346.74	0.004	6
91-10-1	1,3-Dimethoxypyrogallol	-	1.10[c]	12.59	-	6
620-18-8	3-Vinylphenol	-	2.20[c]	158.49	-	6

[§] All concentrations reported in this table were taken from IARC monograph 38 unless otherwise noted by the appropriate reference number listed next to the compound name.

[a] Concentration value representing total of two compounds: 2-Ethylaniline + 2,6 –Dimethylaniline; 3-Ethylaniline + 2,4-Dimethylaniline; 4-Ethylaniline + 2,3-Dimethylaniline; Triphenylene + Chrysene

[c] Indicates a calculated value for Log P