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Fundamental and Molecular Mechanisms of Mutagenesis

Dosimetry, toxicity and carcinogenicity of inspired acetaldehyde in the rat

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Abstract

Acetaldehyde is a ubiquitous air pollutant. It is an important industrial chemical and is also produced during the combustion of wood or tobacco. In smoky indoor atmospheres concentrations of the aldehyde may reach 100 ppb. Acetaldehyde is metabolized to acetate (releasing hydrogen ion) by aldehyde dehydrogenase a process which, in most tissues, represents a detoxification pathway. In vitro, acetaldehyde forms DNA–DNA and DNA–protein crosslinks. It is a clastogen, and inducer of sister chromatid exchanges, and is, perhaps, a weak mutagen. Inhalation exposure to 1000 ppm may induce DNA–protein crosslink formation in nasal tissues in the rat in vivo. Inhalation toxicity studies have shown acetaldehyde vapor causes chronic tissue injury and tumor formation in nasal tissues at exposure concentrations of 750 ppm or higher, with nasal olfactory mucosa being more sensitive than respiratory mucosa. Dosimetric estimates suggest that marked tissue injury and carcinogenicity occurs only at inspired concentrations which are sufficiently high to overwhelm nasal aldehyde dehydrogenase detoxification capacity. The induction of squamous cell carcinomas in the respiratory mucosa by acetaldehyde displays many analogies to the induction of squamous cell carcinomas by formaldehyde. For both vapors, non-linear concentration response relationships are observed for DNA–protein crosslink formation, tissue injury, and carcinogenicity, suggesting these responses are associated. For both vapors it is possible to document an exposure concentration that produces nasal respiratory epithelial injury without increasing tumor incidence, suggesting that for respiratory mucosa-derived tumors, exposure to non-cytotoxic concentrations may pose limited carcinogenic risk. In addition to squamous cell carcinomas of the respiratory epithelium, acetaldehyde exposure also results in nasal olfactory injury and tumors (adenocarcinomas) in the rat. The studies performed to date have not demonstrated a no observable effect level for these responses, therefore, the precise role of cytotoxicity and regenerative cell proliferation in the carcinogenic process in olfactory tissues can not be evaluated. Acetaldehyde metabolism via aldehyde dehydrogenase results in the formation of two hydrogen ions. The olfactory mucosa is quite sensitive to acid and dosimetric estimates suggest that the intracellular acid production rates that may occur in olfactory mucosa during acetaldehyde exposure may be sufficiently high to cause tissue damage. Such acid-induced tissue damage may enhance the genotoxic and tumorigenic potential of acetaldehyde in olfactory mucosa, and may, therefore, represent an important process in the production of tumors in this tissue. © 1997 Elsevier Science B.V.

Keywords: Acetaldehyde; Carcinogenicity; Toxicity; Inhalation; Rat

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1. Introduction

Acetaldehyde (ethanal) is a low-molecular-weight, saturated reactive aldehyde. It is an important industrial chemical that is used in the plastics and resin industry, the paper industry, and for the synthesis of organic chemicals. Industrial emissions of this aldehyde in 1993 were estimated to be in excess of 9 000 000 pounds (TRI, 1993). Acetaldehyde is a common indoor air pollutant, being produced during the combustion of wood or cigarettes. It may be the aldehyde of highest concentration in tobacco smoke (Houlgate et al., 1989; Lofroth et al., 1989). Concentrations in smoky atmospheres can achieve 100 ppb (Badre et al., 1978). Acetaldehyde is also present naturally in many foods (see Feron et al., 1991). Finally, acetaldehyde is a major toxic metabolite of ethanol.

The focus of the current review is on the inhalation toxicity of acetaldehyde in the rat. Thus, the extensive literature on acetaldehyde as a critical component of ethanol toxicity will not be reviewed, nor will the data on oral administration of this compound. The inhalation toxicity and mutagenicity of acetaldehyde vapor has been recently reviewed by Feron et al. (1991) and Dellarco (1988). Rather than re-review this information the reader will be referred to these sources. In addition, due to the extensive similarity of the chemistry and toxicity of acetaldehyde and formaldehyde, the reader is also referred to recent reviews on formaldehyde (Heck et al., 1990; Monticello and Morgan, 1994).

Acetaldehyde is classified as a B2 probable human carcinogen by the US Environmental Protection Agency (EPA) (IRIS, 1996) based on the increased incidence of nasal tumors in rats and laryngeal tumors in hamsters after chronic inhalation exposure. Exposure of hamsters to 2000 ppm (7 h/day, 5 days/week) for 52 weeks with a 29-week recovery period resulted in a slight increase in nasal tumors and a significant increase in laryngeal tumors (Feron et al., 1982). Acetaldehyde exposure also enhanced the tumorigenicity of benzo[*a*]pyrene in the hamster respiratory tract indicating it can act as a promoter (Feron, 1979; Feron et al., 1982). In the rat, nasal tumors are induced by lifetime exposure to concentrations of 750 ppm or higher (Woutersen et al., 1986). Human data on acetaldehyde carcinogenicity were considered inadequate by the EPA (IRIS, 1996). Acetaldehyde exposure produces non-carcinogenic injury including degeneration and hyperplasia in the rat respiratory tract, with the nasal cavity being the primary target (see below). The nasal olfactory mucosa is considerably more sensitive to the effects of acetaldehyde than the respiratory mucosa.

This article will briefly review the biochemistry, genotoxicity, toxicity (non-carcinogenic) and carcinogenicity of acetaldehyde. Special emphasis will be placed on examination of dose–response relationships. As these relationships are best defined for the rat, the focus of this review will be on this species. In recognition of the fact that nasal respiratory and olfactory mucosa represent two tissues which are known to have differing sensitivities to a variety of inhaled toxicants, the effects of acetaldehyde on these tissues will be considered separately. So doing provides additional insights into the toxicity and carcinogenicity of this compound.

2. Biochemistry and genotoxicity

2.1. Biochemistry

Acetaldehyde reacts with amine and sulfhydryl groups (Dellarco, 1988; Feron et al., 1991). In this regard acetaldehyde is qualitatively similar to formaldehyde (Heck et al., 1990). By analogy to formaldehyde potential sites of reaction of acetaldehyde with DNA include the exocyclic amino groups of deoxyguanosine, deoxycytidine and deoxyadenosine and the endocyclic imino group of thymidine (Heck et al., 1990). As discussed below, in vitro and in vivo studies have suggested that acetaldehyde can form DNA–DNA and DNA–protein crosslinks (Dellarco, 1988; Grafstrom et al., 1994; Lam et al., 1986; Kuykendall and Bogdanffy, 1992).

Acetaldehyde is metabolized to acetate with release of two hydrogen ions by acetaldehyde dehydrogenase (AldDH, EC 1.2.1.3), a process which, in most tissues, represents a detoxifying pathway (Lindahl, 1992). Casanova-Schmitz et al. (1984) have characterized acetaldehyde metabolism kinetics in vitro in homogenates of nasal tissues of the F344 rat. Two isozymes were detected in both respiratory and olfactory mucosa: a high affinity–low capacity and

low affinity–high capacity form. These studies utilized $9000 \times g$ supernatants, thus mitochondrial activity would not have been detected. Studies in our laboratory (unpublished data), however, have revealed that AldDH activity in rodent nasal homogenate $9000 \times g$ pellets is 7-fold or more lower than $9000 \times g$ supernatants suggesting that, compared to cytosol, little mitochondrial activity is present in nasal tissue.

In both respiratory and olfactory mucosa the K_m values for the low affinity–high capacity isozymes are approximately 20 mM (Casanova-Schmitz et al., 1984). The V_{max} values are approximately 130 and 30 nmol/min/mg protein in respiratory and olfactory mucosa, respectively. Assuming the respiratory and olfactory mucosa of the F344 rat nose each contain 8 mg of protein (Casanova-Schmitz et al., 1984), these values correspond to approximately 1000 and 250 nmol/min/tissue. Thus, the total metabolic capacity for the entire nasal cavity for this isozyme is 1250 nmol/min. K_m values for the high affinity–low capacity isozymes are less than 0.2 mM (Casanova-Schmitz et al., 1984). Reported V_{max} values were 0.8 and 2.2 nmol/min/mg protein, corresponding to 6 and 18 nmol/min/tissue for the respiratory and olfactory mucosa, respectively. Thus, it is clear that the respiratory mucosa contains much higher levels of AldDH than the olfactory mucosa. This may explain, in part, the increased resistance of the respiratory mucosa to the toxic and carcinogenic effects of acetaldehyde (Bogdanffy et al., 1985). Although important at low substrate concentrations, the high affinity–low capacity isozymes would not be expected to contribute significantly to total nasal acetaldehyde metabolism at high substrate concentrations due to the low V_{max} values.

Histochemical studies of AldDH in the respiratory tract of the F344 rat have been performed by Bogdanffy et al. (1985). These studies revealed that the enzyme was widely distributed throughout the cells of the respiratory epithelium with the highest levels being observed in ciliated epithelial cells. Lower levels were detected in olfactory mucosa, with the only epithelial cells demonstrating significant activity being the basal cells. A weak to moderate activity was detected in the acinar cells of Bowman's glands. These studies are fully consistent with the in vitro enzyme kinetic studies of Casanova-Schmitz et al. (1984) which showed that AldDH is present in the nasal mucosa of the rat, with the respiratory mucosa possessing the higher metabolic capacity.

2.2. Genotoxicity

In vitro studies have revealed acetaldehyde reacts with nasal DNA to form DNA–protein crosslinks and DNA–DNA crosslinks (Lam et al., 1986; Dellarco, 1988; Kuykendall and Bogdanffy, 1992; Grafstrom et al., 1994). In this regard acetaldehyde displays many qualitative similarities to formaldehyde (Heck et al., 1990). By analogy to formaldehyde, reaction of acetaldehyde may be more rapid with single-stranded than double-stranded DNA (see Lam et al., 1986; Woutersen et al., 1986; Feron et al., 1991). Kuykendall and Bogdanffy (1992) compared the reaction kinetics for aldehyde-induced DNA–histone crosslinking in vitro and found acetaldehyde to be approximately three orders of magnitude less reactive than formaldehyde.

In an extensive review, Dellarco (1988) concluded that acetaldehyde is a clastogen and inducer of sister-chromatid exchanges, but that limited information was available on its ability to cause gene mutations. Since that time, a few studies have examined the mutagenic potential of acetaldehyde in vitro. At concentrations of 10 mM or higher, acetaldehyde was shown to cause significant levels of 6-thioguanine resistance mutations in cultured skin fibroblasts (Grafstrom et al., 1994). However, a clear dose–response relationship was not observed, and 50% cytotoxicity was observed at mutagenic concentrations. Acetaldehyde-induced mutations at the *hprt* locus in human lymphocytes have been observed in vitro at concentrations of 0.2 mM or greater (He and Lambert, 1990). The increase in mutation frequency was small leading the authors to conclude that acetaldehyde was a comparatively weak mutagen in the system that was utilized. In summary, considerable evidence suggests that acetaldehyde is a clastogen (see Dellarco, 1988). Incomplete data are available on the potential mutagenic activity of acetaldehyde in mammalian cells.

The interactions of inhaled acetaldehyde with nasal DNA of the F344 rat have been examined by Lam et al. (1986). These studies used an indirect measure of DNA–protein crosslink formation — the

formation of ''interfacial DNA'' based on a chloroform/isoamyl alcohol/phenol extraction technique. This measure has been utilized and validated for formaldehyde-induced crosslink formation (e.g. see Heck et al., 1990), but extensive studies have not been performed with acetaldehyde. Nevertheless, examination of the interfacial DNA response leads to interesting insights and also allows comparisons of the potency of acetaldehyde to that of formaldehyde.

Single 6-h inhalation exposure to 1000 or 3000 ppm acetaldehyde resulted in concentration-dependent increases in nasal respiratory mucosal DNA–protein crosslinks as measured by an increase in percent interfacial DNA. Exposure to 100 ppm was without apparent effect, whereas exposure to 300 ppm increased the percent interfacial DNA, but the effect was not statistically significant. Thus, the concentration–response curve for this effect is non-linear. Formaldehyde also demonstrates a non-linear concentration–response curve for DNA protein crosslink formation (Casanova et al., 1989; Heck et al., 1990) On a quantitative basis, exposure to 1000 ppm acetaldehyde produced approximately the same level of respiratory mucosa interfacial DNA as exposure to 5.6 ppm formaldehyde (Casanova-Schmitz and Heck, 1983), confirming the reduced potency of acetaldehyde compared to formaldehyde. Exposure of rats to 1000 ppm acetaldehyde for 6 h/day for 5 consecutive days did not increase the level of interfacial DNA in respiratory mucosa tissues over that observed after 1 day of exposure.

The formation of DNA–protein crosslinks in olfactory mucosa by inhalation exposure to acetaldehyde, as measured by interfacial DNA, displayed different kinetic behavior than for respiratory mucosa. Specifically, although single 6-h exposure to acetaldehyde at concentrations as high as 1000 or 3000 ppm did not increase olfactory tissue interfacial DNA levels, interfacial DNA levels were significantly elevated by repeated exposure (6 h/day, 5 days/week, 1000 ppm). The reasons for the differing kinetic behavior of the olfactory versus respiratory mucosa are not known. They may be dosimetric in origin, and/or formation of DNA–protein crosslinks in olfactory epithelium may be dependent on cytotoxicity. As discussed below, olfactory degeneration is induced by exposure to 400 ppm, thus 1000 ppm would be anticipated to be a cytotoxic concentration of acetaldehyde. By analogy to formaldehyde, acetaldehyde may react preferentially with single-stranded DNA (Lam et al., 1986; Woutersen et al., 1986). Thus, cytotoxicity and increased regenerative cell proliferation may enhance DNA–protein crosslink formation. If so, crosslinks might not be expected after a single exposure due to the low cell turnover in the olfactory epithelium, but might occur after repeated exposure to cytotoxic concentrations of acetaldehyde. Lack of cell proliferation data for acetaldehyde make this hypothesis conjectural. For comparative purposes it is noted that formaldehyde does not form DNA–protein crosslinks in olfactory epithelium (Casanova-Schmitz and Heck, 1983), perhaps due to near total scrubbing of formaldehyde from the airstream in the anterior portion of the nasal cavity (Kimbell et al., 1993).

3. Dosimetry

Nasal dosimetry of acetaldehyde has been measured in studies in this laboratory in young anesthetized male F344 rats (Morris and Blanchard, 1992) in which the upper respiratory tract was isolated via insertion of an endotracheal tube anteriorly into the trachea such that its tip lay at the larynx. (Acetaldehyde deposition was not measured in female rats; however, our previous studies failed to detect a gender difference in nasal vapor deposition Morris et al., 1991.) The acetaldehyde studies utilized constant velocity unidirectional inspiratory flow and a pseudo-cyclic flow regimen (100 ml/min sinusoidal flow maintained by a rodent respirator pump with 7 ml/min continuous bleed for analysis). Deposition was measured at inspired concentrations of 1, 10, 100 and 1000 ppm.

These studies revealed that deposition efficiency was highly dependent on air flow rate and on inspired concentration. Diminished deposition efficiency was observed with increasing air flow rates. This a generalized phenomenon that has been observed for numerous vapors in our laboratory (Morris, 1990, 1993, 1994). Under all flow conditions acetaldehyde deposition efficiencies decreased with increasing inspired concentration. For example, under the pseudo-cyclic flow conditions, deposition efficiencies averaged 76%, 48%, 41% and 26% at

inspired concentrations of 1, 10, 100 and 1000 ppm, respectively. The mechanism for the non-linear deposition is unknown. It was postulated reduced deposition at high concentrations was related to overwhelming of the nasal metabolic capacity for acetaldehyde. This conjecture was based on other studies which had shown nasal metabolism serves to increase nasal vapor deposition efficiencies of carboxylesterase (Morris, 1990), alcohol dehydrogenase, and mixed function oxidase (Morris, 1993) substrate vapors. In addition, pretreatment with an AldDH inhibitor reduced nasal acetaldehyde deposition rates (Morris and Blanchard, 1992). As outlined below, comparisons of the deposition rate (μg/min) with the total nasal AldDH metabolic capacity also suggested that metabolism was capacity-limited at the high exposure concentrations in this study.

Nasal deposition efficiencies of acetaldehyde in normally breathing animals are not known. Our studies used an anesthetized animal and non-physiologic flow conditions (Morris and Blanchard, 1992). However, if the efficiencies measured under the pseudocyclic flow conditions are used as a first approximation, then delivered dosage rates can be estimated for various exposure concentrations. Assuming a ventilation rate of 200 ml/min for adult rats in a chronic inhalation study, animals exposed to 100 or 1000 ppm inhaled 0.8 and 8 μmol/min, respectively, of acetaldehyde. Assuming total nasal cavity deposition efficiencies of 41% and 26%, delivered dosage rates of 0.3 and 2 μmol/min are estimated. The total nasal AldDH metabolic capacity was estimated to be 1.3 μmol/min (see above). Thus, at 1000 ppm this capacity, at least on a whole-nose basis, would be expected to be exceeded. Assuming 26% deposition and 200 ml/min ventilation rates, deposited dosage rates of 1.3 μmol/min would be predicted at inspired concentrations of 650 ppm. It should be recognized that this is a crude first approximation and is based on total nasal deposition, not regional deposition within the respiratory or olfactory mucosa. For comparative purposes it is noted that for formaldehyde (which is thought to deposit only in the anterior respiratory epithelium, Kimbell et al., 1993), deposited dose rates at approximately 5 ppm (200 ml/min, 93% deposition, Patterson et al., 1986) would be predicted to approximate the nasal formaldehyde dehydrogenase metabolic capacity (40 nmol/min for the respiratory epithelium, Casanova-Schmitz et al., 1984).

The above estimates for acetaldehyde dosimetry are based on a whole nasal cavity basis because regional dosimetry within the nose is not known. Non-uniform deposition would be predicted because the nasal cavity is not uniformly ventilated (Kimbell et al., 1993). All inspired air passes over the anterior respiratory mucosa of the rat nose, however, only a fraction of the airstream passes over the posterior olfactory mucosa. The elegant flow dynamic modeling work of Kimbell et al. (1993) and our PBPK modeling of metabolized vapor deposition (Morris, 1993) suggest only 10% of the inspired airstream passes over the olfactory epithelium. Thus, olfactory dosage rates can not exceed 10% of the inspired burden. Based on the high surface area covered by the olfactory mucosa (approximately 50% of the total nasal surface area in the rat, Gross et al., 1982) and the low flow rates (i.e. 10% of the total), it would be predicted that the relative deposition efficiency over the olfactory epithelium is much higher than that over the respiratory epithelium. A total nasal cavity deposition of 26% (Morris and Blanchard, 1992) might reflect a relative dosage of 10% of the inspired burden in olfactory tissues (near complete deposition from the 10% of the airflow penetrating over that region) and 16% deposition of the inspired burden over the respiratory mucosa. Using these estimates and the tissue-specific metabolism rates (Casanova-Schmitz et al., 1984; see above) it would be estimated that dosage rates in the respiratory epithelium would approximate the local V_{max} value at a concentration of 800 ppm. For olfactory tissue the local V_{max} value would be approximated at an inspired concentration of 300 ppm. Neither of these values differs dramatically from that estimated above on a whole nasal cavity basis.

As discussed above, acetaldehyde DNA–protein crosslinks in rat respiratory mucosa are significantly elevated at exposure concentrations of 1000 but not 300 ppm. This would appear to correlate with the inspired concentration estimated to approximate the AldDH metabolic capacity, viz. 650 ppm. Thus, concentrations less than 650 ppm do not cause statistical increases in crosslink levels whereas higher concentrations do. Non-linear concentration response curves for formaldehyde-induced crosslinks have

been observed, with the slope of the curve being much greater for concentrations from 2–10 ppm compared to 0–2 ppm (Casanova et al., 1989). Interestingly, the dosimetric estimates provided above suggest that dosage rates at an exposure concentration of 5 ppm would approximate the metabolic capacity for formaldehyde detoxification. Thus, enhanced formaldehyde DNA protein crosslink formation occurs at delivered dose rates estimated to exceed the metabolic capacity. Heck et al. (1990) has postulated that the non-linear concentration response curve for formaldehyde-induced DNA–protein crosslinks may be due, in part, to saturation of defense mechanisms. These dosimetric comparison suggest a similar phenomenon may contribute to the non-linear relationship between acetaldehyde exposure concentration and induction of respiratory mucosal DNA–protein crosslinks.

4. Inhalation toxicity

4.1. General effects

Acute, subchronic and chronic exposure to acetaldehyde produces respiratory tract injury in the rat with the most severe damage being produced in the nasal cavity. The 4-h LC_{50} in the rat is 13 300 ppm (Appelman et al., 1982). Rats exhibited severe mouth breathing during the exposure suggesting occlusion of the nasal passages.

Repeated exposure (6 h/day, 5 days/week, 4 weeks) of rats to 400, 1000, 2200 or 5000 ppm produced marked injury to the nose (Appelman et al., 1982). Degeneration of olfactory nasal tissues was observed at all concentrations. Tracheal and laryngeal lesions were observed at the 2200 and 5000 ppm concentrations only, and mild injury to the lower respiratory tract was observed only at the highest exposure level. Subchronic (13, 26 or 52 weeks) and chronic (28 months) exposure of the rat produce injury to the nasal mucosa and larynx without apparent injury to the tracheobronchial tree or lungs (Woutersen et al., 1984; Woutersen et al., 1986). Marked gender differences were not observed for any of these responses. Thus, in both the male and female rat, the nose is the most sensitive target site.

Results of carcinogenicity studies also indicate the nose is the primary target for acetaldehyde in the rat. Specifically, 28-month exposure to this vapor produces nasal tissue damage (hyperplasia, metaplasia) and tumors in the rat without producing injury in lower respiratory tract tissues (Woutersen et al., 1986). Marked gender differences were not observed for these responses. Two nasal tumor types were observed; squamous cell carcinoma and adenocarcinoma. Interestingly, nasal tumors developed during a 52-week post-exposure period in rats exposed to acetaldehyde for 52 weeks, suggesting that progression of the lesions can occur in the absence of continued exposure (Woutersen and Feron, 1987). The authors concluded that acetaldehyde-induced adenocarcinomas were of olfactory tissue origin (Woutersen et al., 1986). The squamous cell carcinomas were thought to be of primarily respiratory epithelial origin; however, some may have arisen from olfactory mucosa. For the purposes of the current review, the adenocarcinomas will be assumed to be of olfactory origin, while the squamous cell carcinomas will be assumed to be of respiratory epithelial origin. This assumption may overestimate the carcinogenic risk to the respiratory mucosa and underestimate that to the olfactory.

In summary, a consistent finding in all inhalation toxicity studies is that the nasal cavity is the most sensitive target site for inhaled acetaldehyde-induced injury. Toxic responses were documented in both of the principal tissue types of the nose; respiratory and olfactory mucosa. The nasal responses are discussed in detail below. Since these tissues demonstrated differing responsiveness to acetaldehyde, lesions in these two tissues are discussed separately. Responses of both genders are considered as a whole as marked gender differences have not been observed.

4.2. Respiratory mucosal effects

Although the olfactory mucosa is the most sensitive target in the rat for inhaled acetaldehyde, exposure to high concentrations of this vapor produces damage to the respiratory epithelium as well. Degenerative, hyperplastic and carcinogenic responses have been documented in acute, subchronic and/or chronic exposure studies. Fig. 1 displays concentration–response curves for the various responses of the rat nasal respiratory epithelium to acetaldehyde.

Repeated exposure (6 h/day, 5 days/week) of the Wistar rat to acetaldehyde for 4 weeks results in respiratory epithelial degeneration with or without hyperplasia and/or metaplasia (Appelman et al., 1982). Specifically, exposure to 5000 ppm resulted in severe degeneration with hyper/metaplasia in 100% of the animals (see Fig. 1A). Slight to moderate degeneration without hyperplasia or metaplasia was observed in approximately 40% of the animals exposed to 1000 or 2200 ppm. With respect to respiratory mucosal damage, 400 ppm represented a no observable adverse effect level.

Woutersen et al. (1984, 1986) performed a 28-month (lifetime) exposure of the Wistar rat using exposure concentrations of 750, 1500 and 3000/1000 ppm. The exposure protocol for the highest concentration was quite complicated due to severe toxicity. Animals were exposed to 3000 ppm from day 0 to 141, after which time exposure concentrations were slowly reduced to 1000 ppm (by day 359) and maintained at that level to the termination of the study. Interim sacrifices were scheduled after 13, 26 and 52 weeks of exposure (Woutersen et al., 1984). Degeneration of the respiratory epithelium was observed in only the highest concentration group in the interim sacrifice animals. Precise incidences of damage were not tabulated in the description of the interim sacrifice data, thus comprehensive concentration–response curves cannot be prepared.

Complete information on the lesions produced by 28-month exposure to 750, 1500 or 3000/1000 ppm acetaldehyde are provided in Woutersen et al. (1986). Squamous metaplasia (with or without keratinization) was observed in approximately 40% of the animals exposed to 1500 or 3000/1000 ppm (see Fig. 1B). Simple or pseudo-epitheliomatous respiratory epithelial hyperplasia was also observed in a large fraction of animals exposed to these concentrations (Fig. 1B). Exposure to 750 ppm may represent an apparent lowest observable effect level. Specifi-

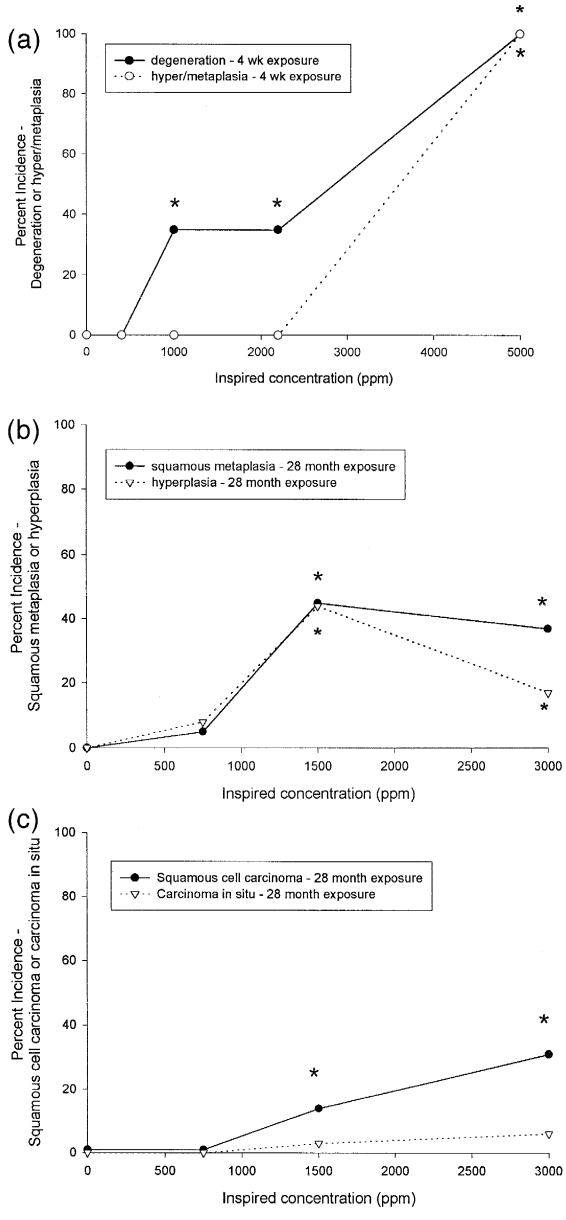

Fig. 1. A: incidence of respiratory epithelial degeneration (closed circles), and hyperplasia or metaplasia (open circles) in rats exposed to 400, 1000, 2200 or 5000 ppm daily for 4 weeks (Appelman et al., 1982). Incidence rates statistically different from control are indicated by the asterisks. B: incidence of respiratory epithelial squamous metaplasia (closed circles), and hyperplasia (open circles) in rats exposed to 750, 1500 or 3000/1000 ppm daily for 28 months (Woutersen et al., 1984). Incidence rates statistically different from control are indicated by the asterisks. C: incidence of respiratory epithelial squamous cell carcinoma (closed circles), and carcinoma in situ (closed circles) in rats exposed to 750, 1500 or 3000/1000 ppm daily for 28 months (Woutersen et al., 1986). Incidence rates statistically different from control are indicated by the asterisks.

cally, squamous metaplasia (without keratinization) or simple hyperplasia was observed in approximately 10% of the animals. The incidence of these observations was not statistically different from controls (Fisher exact test), however, there was 0% incidence of these lesions in controls suggesting, that a minimal biological response may have occurred in this exposure group. The mechanism(s) for acetaldehyde-induced respiratory mucosal injury are not known.

Squamous cell carcinoma incidence was significantly elevated over control levels in animals exposed to 1500 or 3000/1000 ppm acetaldehyde with an apparent concentration–response relationship being observed (Woutersen et al., 1986). The tumor incidences are shown in Fig. 1C. Also observed were carcinomas in situ, although the incidence of this lesion was not significantly elevated over control levels in any exposure group. One squamous cell carcinoma was observed in control rats and one was observed in a rat exposed to 750 ppm acetaldehyde. In both animals the tumor was associated with a dystrophic incisor tooth. These tumors were considered not related to acetaldehyde. Thus, with respect to induction of squamous cell carcinoma, 750 ppm represents a no-observable effect level.

Comparison of the concentration effect relationships for subchronic tissue damage (Fig. 1A), chronic tissue injury (Fig. 1B) and tumorigenic response (Fig. 1C) suggests qualitative similarities between these responses. For all responses, similar non-linear relationships were observed, suggesting the responses are associated. In this regard, many similarities exist between acetaldehyde and formaldehyde (e.g. see Heck et al., 1990; Feron et al., 1991). For example, a 2-fold increase in acetaldehyde exposure concentration (750 to 1500 ppm) resulted in an approximately 15-fold increase in tumor incidence. This is similar to formaldehyde, for which an approximately 3-fold increase in exposure concentration (5.6 to 14.3 ppm) produced an approximately 40-fold increase in tumor incidence.

Acetaldehyde-induced tumors were only observed in animals exposed to possibly cytotoxic levels of this vapors. Specifically, exposure to 1500 ppm elevated tumor rates and also produced a cytotoxic response as indicated by hyperplasia and/or metaplasia. Importantly, exposure to 750 ppm may have produced mild injury but did not elevate tumor rates. Formaldehyde exhibits similar relationships. Exposure to 5.6 ppm formaldehyde induced cytotoxicity but did not elevate tumor incidence (Kerns et al., 1983). It should be pointed out that 5.6 ppm formaldehyde produced more severe respiratory squamous metaplasia than did 750 ppm acetaldehyde (Kerns et al., 1983; Woutersen et al., 1986). Nevertheless the overall relationships appear to be similar for both vapors. Thus, as for formaldehyde, it can be reasonably hypothesized that acetaldehyde-induced cytotoxicity plays a critical role in respiratory epithelial tumor formation. Unfortunately, cell proliferation data such as those for formaldehyde (Monticello and Morgan, 1994) are not available for acetaldehyde. Thus a rigorous examination of this hypothesis is not possible.

It is interesting to compare the estimated tissue dosage rates at the tumorigenic and non-tumorigenic exposure concentrations. As outlined above, acetaldehyde dosage rates at exposure concentrations of approximately 650 ppm would appear to approximate the total nasal metabolic detoxification capacity via AldDH. Interestingly for all four responses, crosslinks (see above), subchronic and chronic tissue damage, and carcinogenicity, exposure concentrations of 750 ppm or less were associated with minimal to injury whereas higher concentrations produced marked damage. Similarly, formaldehyde carcinogenicity is only documented at inspired concentrations above 5 ppm: the estimated exposure concentration at which tissue dosage rate are estimated to approximate the metabolic detoxification capacity of the nose. Thus, saturation of detoxification capacities may provide an explanation for the shape of the concentration response curves for both acetaldehyde and formaldehyde. Heck et al. (1990) have suggested that detoxification pathway saturation may also be important in determining the formaldehyde concentration response relationships. The concentration–response relationships described above are fully consistent with this hypothesis.

Much literature has focused on potential mechanisms for the enhanced carcinogenicity of formaldehyde at cytotoxic versus non-cytotoxic exposure levels (see Heck et al., 1990; Monticello and Morgan, 1994). Possible mechanisms include enhanced reactivity of aldehydes with single-stranded versus

double-stranded DNA, decreased time for repair of DNA lesions with cytotoxicity-induced enhanced regenerative cell proliferation, and/or cell proliferation dependent clonal expansion of initiated cells (see Woutersen et al., 1986; Lam et al., 1986;Heck et al., 1990). Given the chemical similarity and the similarity of the responses to both formaldehyde and acetaldehyde, it appears reasonable to presume that similar mechanisms may play a role in acetaldehyde-induced nasal respiratory epithelial carcinogenesis. Certainly careful analysis of the responses of the respiratory epithelium of the rat to acetaldehyde suggests considerable analogy to formaldehyde. Thus, as for formaldehyde (Heck et al., 1990; Feron et al., 1991), these results suggest nasal respiratory epithelial cytotoxicity is critical for the tumorigenic response and, therefore, respiratory epithelial carcinogenic risk to non-cytotoxic concentrations of acetaldehyde may be quite low.

4.3. Olfactory mucosal effects

The olfactory mucosa is the most sensitive target for acetaldehyde-induced injury. Degeneration with or without hyperplasia/metaplasia of the olfactory mucosa was induced in Wistar rats exposed to 400, 1000, 2200 or 5000 ppm acetaldehyde (6 h/day, 5 days/week) for 4 weeks (Appelman et al., 1982). Even at the lowest exposure concentration, degeneration (without hyperplasia) was observed in 80% of the animals. Thus, the study of Appelman et al. (1982) did not demonstrate a no observable effect level for olfactory damage. Subsequent studies revealed that 150 ppm is a no observable effect level of olfactory damage in the rat (Appelman et al., 1986).

Olfactory tissue damage was observed in all exposure groups (750, 1500, 3000/1000 ppm 6 h/day, 5 days/week) in the 28-month exposure study of Woutersen et al. (1986), including the 13, 26, and 52 week interim sacrifice groups (Woutersen et al., 1984). Olfactory tissue cytotoxicity and repair, as indicated by basal cell hyperplasia of the olfactory epithelium was observed in approximately 80% of the animals exposed to 750 ppm acetaldehyde for 28 months. Thus, even the lowest exposure concentration produced a high incidence of tissue damage. Adenocarcinomas were observed in all exposure groups after 28 months of exposure. The incidence of this tumor was 0% in controls compared to approximately 20% in the 750 ppm group and 50% in the 1500 ppm group. As for hyperplasia, a marked tumorigenic response was observed even at the lowest exposure concentration.

In summary, olfactory tumors have been observed in animals exposed to cytotoxic (as indicated by degeneration and hyperplasia) concentrations of acetaldehyde. It is not possible to assess the role of tissue cytotoxicity in the induction of olfactory tumors by acetaldehyde as the experiments performed to date have not documented a cytotoxic level which is not tumorigenic. By analogy to formaldehyde, and also by analogy to the response of the respiratory mucosa to acetaldehyde (see above), it does not seem unreasonable to predict that acetaldehyde-induced cytotoxicity plays an important role in induction of olfactory tumors; however, in the absence of data this supposition is quite speculative.

The mechanisms for the enhanced responsiveness of the olfactory compared to respiratory epithelium for acetaldehyde are not known. However, comparisons of the response to acetaldehyde with that to other olfactory-selective toxicants provides potential insights. AldDH metabolism of acetaldehyde produces acetate with release of two hydrogen ions (Casanova-Schmitz et al., 1984). The olfactory epithelium is quite sensitive to acids. Inhalation exposure to 225 ppm acetic acid (6 h/day, 5 days/week, 2 weeks) produces nasal olfactory but not respiratory epithelial damage (see Stott and McKenna, 1985). Acrylic acid exposure produces similar olfactory epithelial injury (Miller et al., 1981). Thus, production of acetic acid by olfactory AldDH may play a role in the acetaldehyde-induced olfactory damage.

The dose–response relationships for inspired acid may differ from intracellularly generated acid (Frederick et al., 1994; Morris and Frederick, 1995). Ester vapors are metabolized to intracellular acids in nasal mucosa via carboxylesterase (Stott and McKenna, 1985). Dosimetric comparisons of the toxicity of acetaldehyde and ester vapors supports the concept that AldDH generated acid may play a role in acetaldehyde-induced olfactory injury. Carboxylesterase is present throughout the rat nose mucosa with the highest levels being present in the

olfactory mucosa (Bogdanffy et al., 1987). The cellular distribution of carboxylesterase differs somewhat from that of aldehyde dehydrogenase (Bogdanffy et al., 1985; Bogdanffy et al., 1987) and the most ideal comparison of aldehyde and ester metabolism and toxicity would be on a cell-specific basis. Unfortunately, insufficient data are available to allow for such an analysis. Therefore, a more generalized comparison is provided below.

Numerous esters, including propylene glycol monomethyl ether acetate (Miller et al., 1984), ethyl acrylate (Miller et al., 1985), and the dibasic esters dimethyl succinate, dimethyl glutarate and dimethyl adipate (Keenan et al., 1990) have been shown to produce olfactory degeneration via a carboxylesterase-dependent mechanism (Stott and McKenna, 1985; Trela and Bogdanffy, 1991). All of these esters are extensively metabolized in nasal tissues via carboxylesterase. Carboxylesterase represents a high capacity–low affinity pathway as evidenced, for example, by nasal olfactory V_{max} values of approximately 0.2 μmol/min/mg protein for ethyl acrylate and 7 μmol/min/mg protein for dimethyl succinate, and K_m values in the high μM to mM range (Stott and McKenna, 1985; Bogdanffy et al., 1991; Frederick et al., 1994). Olfactory mucosal metabolism of acetaldehyde is of lower capacity (0.03 μmol/min/mg protein); however, the critical factor is that both esters and acetaldehyde are metabolized to acids.

The most complete nasal dosimetric data are available for the dibasic esters and ethyl acrylate. Because ethyl acrylate is directly reactive, its toxicity may not be solely due to acid production (Frederick et al., 1994; Morris and Frederick, 1995). Thus, the most appropriate dosimetric comparisons to assess potential acid-mediated effects would be between acetaldehyde and the dibasic esters. Subchronic exposure to 76 mg/m^3 (~12 ppm) dibasic esters results in olfactory damage in the rat (Keenan et al., 1990). Dibasic esters deposit with near total efficiency in the nose and a major fraction of the deposited dose is thought to be metabolized in situ (Morris et al., 1991). Dibasic ester dosage rates (to the entire nasal cavity) of 0.1 μmol/min would be predicted at an inspired concentration of 76 mg/m^3 assuming a ventilation rate of 200 ml/min, corresponding to acid production rates of 0.1 μmol/min even if the entire deposited burden were metabolized. The estimated total nasal cavity dosage rate for acetaldehyde is 1.3 μmol/min at 650 ppm (see above), suggesting that acetaldehyde dosage rates (on a whole nasal cavity basis) are sufficiently high at inhaled concentrations of 650 ppm or more to produce biologically significant amounts of acid, even if only a small fraction of the deposited dose is metabolized in situ. Unfortunately, insufficient information is available to perform this dosimetric comparison on the basis of delivered dosage to the specific target, the olfactory epithelium. However, if dibasic esters at a concentration of 12 ppm produce olfactory damage as a result of metabolic acid production, then it is possible that acid production rates from acetaldehyde at concentrations which produce olfactory damage (400 ppm or more) may also be sufficient to induce olfactory injury.

Chronic acid-induced olfactory degeneration is not sufficient to produce nasal tumors in the rat as evidenced by the fact that chronic exposure to ethyl acrylate at concentrations which produce olfactory degeneration, hyperplasia and metaplasia, did not induce olfactory tumors (Miller et al., 1984) Therefore, it appears that the genotoxicity of acetaldehyde is also critical for the tumorigenic response. Vinyl acetate, is a nasal carcinogen in the rat and metabolized by nasal carboxylesterases to acetic acid and acetaldehyde (Bogdanffy et al., 1994). Kuykendall et al. (1993) have provided data suggesting that both the acetic acid-dependent cytotoxicity and the genotoxicity of acetaldehyde are critical for the response to vinyl acetate. Such may well be the case for acetaldehyde itself. An integrated hypothesis for olfactory-induced tumorigenesis is that acetaldehyde induces olfactory cytotoxicity and cell proliferation through acid-dependent pathways. By analogy to formaldehyde, this enhances the genotoxic potential of acetaldehyde via increased potential for interaction with single-stranded DNA, decreased time for repair of lesions and/or clonal expansion of initiated cells (Lam et al., 1986; Heck et al., 1990). Thus, both the cytotoxic and genotoxic potential of acetaldehyde may be critical for the tumorigenic effects of this compound, and unlike many other tissues, acetaldehyde dehydrogenase oxidation of acetaldehyde may play a role in enhancing the nasal response to this reactive aldehyde.

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