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The genotoxic air pollutant 3-nitrobenzanthrone and its reactive metabolite *N*-hydroxy-3-aminobenzanthrone lack initiating and complete carcinogenic activity in NMRI mouse skin

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Abstract

3-Nitrobenzanthrone (3-NBA), a genotoxic mutagen found in diesel exhaust and ambient air pollution and its active metabolite *N*-hydroxy-3-aminobenzanthrone (*N*-OH-3-ABA) were tested for initiating and complete carcinogenic activity in the NMRI mouse skin carcinogenesis model. Both compounds were found to be inactive as either tumour initiators or complete carcinogens in mouse skin over a dose range of 25–400 nmol. Topical application of 3-NBA and *N*-OH-3-ABA produced DNA adduct patterns in epidermis, detected by ^{32}P -postlabelling, similar to those found previously in other organs of rats and mice. 24 hours after a single treatment of 100 nmol DNA adduct levels produced by 3-NBA (18 ± 4 adducts/ 10^8 nucleotides) were 6 times lower than those by 7,12-dimethylbenz[*a*]anthracene (DMBA; 114 ± 37 adducts/ 10^8 nucleotides). In contrast, identical treatment with *N*-OH-3-ABA resulted in adduct levels in the same range as with DMBA (136 ± 25 adducts/ 10^8 nucleotides), indicating that initial DNA adduct levels do not parallel tumour-initiating activity. When compounds were tested for tumour initiating activity by a single treatment followed by twice-weekly applications of TPA, DNA adducts formed by DMBA, but not by 3-NBA or *N*-OH-3-ABA, were still detectable 40 weeks after treatment. When tested for activity as complete carcinogens by twice-weekly topical application, 3-NBA and *N*-OH-3-ABA produced identical DNA adduct profiles in mouse skin, with adducts still detectable after 40 weeks. Only 3-NBA produced detectable adducts in other organs.

Keywords: 3-Nitrobenzanthrone; 7,12-dimethylbenz[*a*]anthracene; Carcinogenesis; DNA adduct; Diesel exhaust; Air pollution

1. Introduction

A large body of evidence supports the concept that carcinogenesis is a multistage process and its stages have been defined experimentally as initiation, promotion and progression [1]. Thus, models mimicking this process, especially the multistage mouse skin carcinogenesis model, can serve as powerful methods for the study of cancer induction and chemoprevention [2-4]. Initiation involves mutation of cellular DNA resulting in the activation of proto-oncogenes (*e.g. ras*) and inactivation of tumour suppressor genes [5,6]. Initiation is thought to be irreversible consisting of a single gene mutation caused by environmental genotoxic agents [7]. Clonal expansion of these initiated cells due to promoting stimuli, for example by application of potent phorbol ester-type skin tumour promoters, is the driving force for development of skin papillomas that may develop into squamous cell carcinoma [8]. Currently, the two-stage mouse skin carcinogenesis protocol consists of an initiation phase by the administration of a single low dose of a carcinogen such as 7,12-dimethylbenz[*a*]anthracene (DMBA) and a promotion phase involving repeated application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [9]. This protocol provides a rapid response with relative ease of quantification of various parameters of tumourigenic response, including tumour incidence, latency, multiplicity and malignancy [2]. Moreover, as shown for DMBA [10] this model system offers a rapid approach to investigate distinct molecular mechanisms of tumour development including mutation analysis in critical target genes (*e.g. ras*) for carcinogenesis. The two-stage mouse skin carcinogenesis model has been used not only to examine the carcinogenic potential of single genotoxic agents [11,12] but also to evaluate the cancer risk of complex environmental mixtures such as cigarette smoke condensate, coal tar and diesel exhaust extracts [4,13-16].

Numerous epidemiological studies have found increased mortality and morbidity from respiratory and cardiovascular diseases associated with exposures to ambient air pollution [17,18]. A complex variety of genotoxins has been detected in urban air pollution [19] and high exposures are associated with an increased risk of cancer. Polycyclic aromatic hydrocarbons (PAHs) and nitropolycyclic aromatic hydrocarbons (nitro-PAHs) are present in particulate matter from direct atmospheric emission, such as diesel and gasoline exhaust [19-21]. Nitro-PAHs often have greater mutagenic and carcinogenic properties compared to their parent PAHs, and their persistence in the environment has led to considerable interest in assessing their potential risk to humans [22-24].

The aromatic nitroketone 3-nitrobenzanthrone (3-NBA; 3-nitro-7H-benz[*de*]anthracen-7-one; Fig. 1), identified in diesel exhaust and ambient air pollution [25,26], is one of the most potently mutagenic compounds ever detected in the Salmonella reverse mutation assay, and it is a suspected human carcinogen [25,27]. Its isomer 2-nitrobenzanthrone has also been detected in urban air particulate matter but has a much lower genotoxic potential [28-30]. 3-NBA forms DNA

adducts *in vitro* and in rodents *in vivo* after metabolic activation through reduction of the nitro group, primarily catalysed by cytosolic nitroreductases such as NAD(P)H:quinone oxidoreductase (NQO1) (Fig. 1) [31-35]. The uptake of 3-NBA in humans has been demonstrated by the detection of 3-aminobenzanthrone (3-ABA; Fig. 1), its main metabolite, in the urine of workers occupationally exposed to diesel emissions [36]. The genotoxicity of 3-ABA has been demonstrated in several short-term assays [37,38]. 3-ABA is predominantly activated by cytochrome P450 (CYP) enzymes, namely CYP1A1 and CYP1A2 [39,40]. Both 3-NBA and 3-ABA can be further activated by *N*-acetyltransferases (NATs) and sulfotransferases (SULTs), *N*-hydroxy-3-aminobenzanthrone (*N*-OH-3-ABA; Fig. 1) being the reactive intermediate [33,41,42]. The predominant DNA adducts detected by ³²P-postlabelling *in vivo* in rodents after treatment with either 3-NBA or 3-ABA are 2-(2'-deoxyguanosin-*N*²-yl)-3-aminobenzanthrone (dG-*N*²-ABA) and *N*-(2'-deoxyguanosin-8-yl)-3-aminobenzanthrone (dG-C8-*N*-ABA) [43,44], and these are most probably responsible for the GC→TA transversion mutations induced by 3-NBA exposure *in vitro* and *in vivo* [45,46]. These DNA adducts not only represent premutagenic lesions in DNA, but they may also be of primary importance for tumour development in target tissues [27,47,48]. The mouse skin carcinogenesis model may provide a powerful tool to examine the molecular mechanism(s) involved in tumour initiation by 3-NBA (*e.g.* oncogene activation) and to correlate mutations in critical target genes (*e.g.* *ras* protooncogene) found in the tumours with promutagenic DNA adducts formed by this rodent carcinogen.

In the present study we have explored and assessed 3-NBA and its reactive metabolite *N*-OH-3-ABA for carcinogenicity and tumour initiating activity in mouse skin. In addition, DNA adduct formation was investigated using ³²P-postlabelling, and adduct levels formed by 3-NBA and *N*-OH-3-ABA were compared with those formed by DMBA.

2. Material and methods

2.1. Test Compounds

Caution: 3-NBA is a potent mutagen, rodent carcinogen and suspected human carcinogen. 3-NBA and its derivatives should be handled with extreme care.

3-NBA (CAS No. 17117-34-9) was prepared as described previously and its authenticity was confirmed by UV spectroscopy, electrospray mass spectrometry, and high-field proton NMR spectroscopy [41]. *N*-OH-3-ABA was prepared as reported [49]. 7,12-Dimethylbenz[*a*]anthracene (DMBA) was purchased from Sigma-Aldrich, Germany.

2.2. Animals

NMRI mice (outbred strain from RCC, Füllinsdorf, Switzerland) were kept in the German Cancer Research Center under an artificial day/night rhythm and fed Altromin standard food pellets and water *ad libitum*. All animal experiments were approved by the Governmental Committee for animal experimentation (License 002/98).

2.3. Tumour initiation/complete carcinogenesis

For initiation-promotion experiments, groups of seven-week-old female NMRI mice [8] were treated with a single topical application of 3-NBA, *N*-OH-3-ABA or DMBA (all dissolved in 0.1 ml acetone) or solvent (0.1 ml acetone) only according to Table 1. Beginning 1 week later, mice were treated twice weekly with the promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA, dissolved in 0.1 ml acetone; Sigma, Germany) or solvent (0.1 ml acetone) only according to Table 1 for 30 (experiment I) or 40 weeks (experiment II), respectively. For complete carcinogenicity experiments, NMRI mice were treated with a single topical application of 3-NBA, *N*-OH-3-ABA or DMBA (all dissolved in 0.1 ml acetone) according to Table 2, followed 1 week later by twice weekly applications of 3-NBA, *N*-OH-3-ABA or DMBA for 40 weeks (Table 2; part of experiment II). All agents were applied to the shaved back skin (shaved skin surface: 8 cm²), a single time for initiation and twice weekly for promotion or complete carcinogenesis. Group 1/1 and 2/1 were used as positive control for initiation-promotion and groups 1/2 and 1/3 as negative control according to the standard protocol. Group 3/1 was used as positive control for complete carcinogenesis. The tumour incidence (tumour bearers/survivors) and yield (number of tumours/survivors) were recorded weekly. Tumours were identified first by visual inspection and later by histological analysis [8].

2.4. Short-term animal treatment

For DNA adduct analysis additional short-term treatments were performed. Groups of seven-week-old female NMRI mice ($n = 2$) were treated topically with one, two or four daily applications of 3-NBA, *N*-OH-3-ABA or DMBA (all dissolved in 0.1 ml acetone) and killed either 24 h or 2 weeks after the final treatment. Mice were killed by cervical dislocation after treatment and the back skin was dissected, flattened on a filter paper and immediately frozen on a cold table at -90°C . The frozen epidermis was scraped off with a precooled scalpel under histological control as described [50].

2.5. DNA extraction

Individual epidermal skin scrapings were pulverised in a ball mill at the temperature of liquid nitrogen. The isolation of genomic DNA was achieved using the Blood & Cell Culture DNA kit (Qiagen, Germany), according to the manufacturer's instructions with the modification of the pH to 7.4 and the sodium chloride concentration of elution buffer to 1.4 M. Three internal organs (lung, kidney and pancreas) were removed from animals killed after 40 weeks and stored at -80°C until DNA isolation by standard phenol/chloroform extraction.

2.6. DNA adduct analysis by ^{32}P -postlabelling

DNA adducts were measured for each DNA sample using the ^{32}P -postlabelling method using either nuclease P1 digestion or butanol extraction enrichment [41,51]. Briefly, DNA samples (4 μg) were digested with micrococcal nuclease (120 mUnits) and calf spleen phosphodiesterase (40 mUnits), enriched and labelled as reported. Chromatographic conditions for thin-layer chromatography (TLC) on polyethyleneimine-cellulose (PEI-cellulose) plates (Macherey-Nagel, Düren, Germany) were [41]: D1, 1.0 M sodium phosphate, pH 6; D3, 4 M lithium-formate, 7 M urea, pH 3.5; D4, 0.8 M lithium chloride, 0.5 M Tris, 8.5 M urea, pH 8. After chromatography TLC sheets were scanned using a Packard Instant Imager (Dowers Grove, IL, USA) and DNA adduct levels (RAL, relative adduct labelling) were calculated from adduct cpm, the specific activity of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and the amount of DNA (pmol of DNA-P) used. 3-NBA-derived DNA adducts were identified using authentic standards as described previously [43]. Results are expressed as DNA adducts/ 10^8 nucleotides.

3. Results and discussion

3.1. Tumour initiating and carcinogenic activity

3-NBA was found to be ineffective as tumour initiator in NMRI mouse skin as shown in Table 1 (groups 1/4–1/6). On the other hand the positive control group which received an initiating dose of 100 nmol DMBA had numerous papillomas after 30 weeks (incidence 89%) (Table 1; group 1/1).

N-OH-3-ABA did not induce papilloma formation when mice were initiated with a single dose followed by promotion with TPA for 40 weeks (Table 1; groups 2/2 and 2/3). In the positive control group receiving DMBA as an initiator, the number of papillomas after 40 weeks of treatment was 5.6/mouse (Table 1; group 2/1).

When tested as complete carcinogens, both compounds were found to be inactive (Table 2; groups 2/5 and 2/6). In the positive control group receiving DMBA, the number of papillomas after 40 weeks of treatment was 3.0/mouse (Table 2; group 3/1).

3.2. DNA adduct analysis

In order to gain insight into the mechanisms how DNA adduct formation triggers tumourigenesis epidermal DNA from mice treated with DMBA, 3-NBA and *N*-OH-3-ABA was analysed by the ^{32}P -postlabelling method. DNA adducts were detected and quantified using both enrichment versions of the ^{32}P -postlabelling method: nuclease P1 digestion for the analysis of DMBA-derived adducts, and butanol extraction for adducts derived from 3-NBA or *N*-OH-3-ABA.

3.2.1. Initiation/promotion experiment

After topical treatment with the initiating dose of DMBA (100 nmol) an adduct pattern similar to that found previously in fetal mouse cell culture was observed [52] and mouse skin [10]. It consisted of two areas of intense adduct spots which have been shown to represent DMBA-DNA adducts formed by bay-region diol epoxides. As shown in Figure 2, 24 hours after treatment, the time of maximum DNA binding [53], DMBA produced a total DNA adduct level of 114 ± 37 per 10^8 nucleotides consistent with results obtained before [54]. After 2 weeks the level of total DMBA-DNA adducts had decreased to 30%.

In contrast, total DNA adduct levels formed after 24 hours in the skin by a single dose of 100 or 400 nmol of 3-NBA were six times lower (18 ± 4 and 18 ± 5 per 10^8 nucleotides, respectively), assuming that the labelling efficiency of DMBA- and 3-NBA-derived DNA adducts

are similar. In this context, it is noteworthy that both DMBA and 3-NBA form DNA adducts with adenine and guanine [43,52]. All 3-NBA-DNA adducts detected by ^{32}P -postlabelling in mouse skin were identified as products formed through nitroreduction indicating that mouse skin contains nitroreductases capable of activating 3-NBA. This is in line with the observation of Long and coworkers [55] that NQO1, the principal enzyme for metabolic activation of 3-NBA [33], is active in mouse skin. Nevertheless, our results indicate that bioactivation of 3-NBA by NQO1 in mouse skin occurs to a lesser extent than the activation of DMBA by CYPs.

Treatment with a single dose of *N*-OH-3-ABA (100 nmol) generated the same adducts in epidermal DNA as the parent compound 3-NBA; however, the total level was 7.5 times higher (136 ± 25 per 10^8 nucleotides) and in the same range as for DMBA (114 ± 37 per 10^8 nucleotides) (Figure 3). A 4-times higher dose of *N*-OH-3-ABA, which was also used in the initiation/promotion experiment, increased the adduct level only moderately (184 ± 72 per 10^8 nucleotides). No difference in adduct level was observed between treatment with 100 and 400 nmol 3-NBA. Although DNA adduct levels formed in mouse skin by *N*-OH-3-ABA were comparable to those formed by DMBA, *N*-OH-3-ABA (as opposed to DMBA) was inactive as an tumour initiator suggesting that high initial DNA adduct levels are not sufficient to initiate tumour formation in the mouse skin model.

As shown in Figure 4A, when the three compounds (3-NBA, *N*-OH-3-ABA and DMBA) were used as initiators (single dose of 100 nmol or 400 nmol) followed by weekly doses of TPA for 40 weeks, only DMBA-DNA adducts were detectable in mouse skin at the end of the treatment. This finding is consistent with results reported before by Randerath and colleagues [56,57] documenting the persistence of DMBA-DNA adducts in mouse epidermis even after promotion by TPA. Although 3-NBA-DNA adducts were found to persist in non-proliferating tissues of rats like kidney and lung [48] for 36 weeks after intratracheal administration of a single dose of 3-NBA, no adducts were detectable in mouse skin under promotion conditions (data not shown) suggesting that DNA adduct persistence in target tissues is important for tumour formation.

3.2.2. Complete carcinogenesis experiment

To simulate the early phase of the complete carcinogenesis experiment, mice were treated with 3-NBA and *N*-OH-3-ABA once daily for 2 or 4 days and DNA adduct formation was analysed by ^{32}P -postlabelling. For comparison mice were treated in the same way with DMBA. As shown in Figure 3 multiple treatments with 3-NBA did not lead to a significant increase in DNA adduct levels indicating a saturation of the activation pathways in skin. After 4 daily doses of 100 nmol and

400 nmol *N*-OH-3-ABA adduct levels were similar (201 ± 24 and 198 ± 39 per 10^8 nucleotides, respectively) but 30% lower than those found after identical treatment with DMBA.

After 40 weeks DNA adduct levels generated by 3-NBA and *N*-OH-3-ABA were comparable (14 ± 8 and 15 ± 8 per 10^8 nucleotides, respectively) although *N*-OH-3-ABA was given at a 4-times higher dose (Fig. 4A) indicating that adducts in skin did not accumulate; instead, a steady state level between formation and loss or repair was reached. At the 40-week time-point DNA adduct formation in lung, kidney and pancreas was observed only in animals treated with 3-NBA and not in those treated with *N*-OH-3-ABA (Fig. 4B), suggesting that the metabolite was not transported through the blood to other organs.

3.3. Conclusions

Previous studies showed a weak but significant carcinogenic potential of diesel exhaust extracts in mouse skin although tumourigenic responses varied widely between different diesel samples [16,58]. Although mouse skin has been reported to be sensitive to the tumourigenic effects of PAHs [15] the results of the present study are in line with the general observation that nitro-PAHs show a weaker response in this experimental model. Among the nitro-PAHs present in diesel exhaust that have been studied only 6-nitrochrysene exhibits a clear tumour initiating activity in mouse skin [59]. Dinitropyrenes showed a very weak carcinogenic activity [12] whereas 1-nitropyrene [12], 2-nitrofluorene [60] and 6-nitrobenzo[*a*]pyrene [59] were inactive in mouse skin. A possible explanation for the lack of tumours induced by nitro-PAHs compared to PAHs in mouse skin could be differences in metabolism. Investigations on DNA adduct formation (as a measure for bioactivation) in mouse skin has been limited to certain PAHs [14,16]. Our results indicate that relatively high DNA adduct levels formed in mouse skin may confer initiating activity to PAHs such as DMBA and that the relative low DNA adducts levels induced by 3-NBA account for the lack of initiating activity of nitro-PAHs like 3-NBA. Moreover, our study shows that comparatively large initial DNA adduct levels formed in mouse skin by the reactive metabolite *N*-OH-3-ABA are still not sufficient to confer initiating activity in skin, and suggest that the persistence of DNA adducts in target tissue may also be crucial for tumour development. Indeed, 3-NBA is carcinogenic in rats after intratracheal administration inducing squamous cell carcinomas in the lung [27] and recent studies indicated that both higher initial levels of DNA adducts and their persistence in the target organ contribute to 3-NBA lung tumour development [47,48].

In general nitro-PAHs have been found to have only weak activity in the mouse skin tumour model, one of a number of models used for human risk assessment. This is especially important when considering the potent carcinogenic effects of nitro-PAHs in other carcinogenicity assays

[23]. The mouse skin tumour model is not a sensitive model to examine the carcinogenic potential of diesel exhaust or other complex mixtures containing significant amounts of nitro-PAHs.

Conflict of interest statement

The authors of this study have no conflict of interest or any financial disclosures to make.

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Table 1: Tumour initiating activity of 3-NBA, *N*-OH-3-ABA and DMBA.

Experiment I											
Group number	Initiation (initiator dose)	Promotion (promoter dose)	Group size	Tumour yield (papilloma/surviving animal)				Tumour incidence (% tumour bearers/survivors)			
				12 wks	18 wks	24 wks	30 wks	12 wks	18 wks	24 wks	30 wks
1/1	DMBA (100 nmol/0.1 ml acetone)	TPA (5 nmol/0.1 ml acetone)	20	0.5	6.0	7.7	8.2	50	80	85	89
1/2	DMBA (100 nmol/0.1 ml acetone)	acetone (0.1 ml)	20	0	0	0	0	0	0	0	0
1/3	acetone (0.1 ml)	TPA (5 nmol/0.1 ml acetone)	20	0	0	0	0	0	0	0	0
1/4	3-NBA (25 nmol/0.1 ml acetone)	TPA (5 nmol/0.1 ml acetone)	20	0	0	0	0	0	0	0	0
1/5	3-NBA (100 nmol/0.1 ml acetone)	TPA (5 nmol/0.1 ml acetone)	20	0	0	0	0	0	0	0	0
1/6	3-NBA (400 nmol/0.1 ml acetone)	TPA (5 nmol/0.1 ml acetone)	20	0	0	0	0	0	0	0	0
1/7	3-NBA (400 nmol/0.1 ml acetone)	acetone (0.1 ml)	20	0	0	0	0	0	0	0	0
Experiment II											
Group number	Initiation (initiator dose)	Promotion (promoter dose)	Group size	Tumour yield (papilloma/surviving animal)				Tumour incidence (% tumour bearers/survivors)			
				12 wks	18 wks	24 wks	40 wks	12 wks	18 wks	24 wks	40 wks
2/1	DMBA (100 nmol/0.1 ml acetone)	TPA (5 nmol/0.1 ml acetone)	20	0.9	2.9	3.7	5.6	40	70	74	81
2/2	<i>N</i> -OH-3-ABA (100 nmol/0.1 ml acetone)	TPA (5 nmol/0.1 ml acetone)	20	0	0	0	0.1 [#]	0	0	0	6
2/3	<i>N</i> -OH-3-ABA (400 nmol/0.1 ml acetone)	TPA (5 nmol/0.1 ml acetone)	20	0	0	0	0	0	0	0	0
2/4	<i>N</i> -OH-3-ABA (400 nmol/0.1 ml acetone)	acetone (0.1 ml)	20	0	0	0	0	0	0	0	0

[#] The papillomas observed were well within the historical control range and considered not significant as no papillomas were found in group 2/3.

Table 2: Complete carcinogenic activity of 3-NBA, *N*-OH-3-ABA and DMBA.

Group number	Test compound (dosing)	Group size	Tumour yield (papilloma/surviving animal)				Tumour incidence (% tumour bearers/survivors)			
			12 wks	18 wks	24 wks	40 wks	12 wks	18 wks	24 wks	40 wks
2/5	3-NBA (100 nmol/0.1 ml acetone)	20	0	0	0	0	0	0	0	0
2/6	<i>N</i> -OH-3-ABA (400 nmol/0.1 ml acetone)	20	0	0	0	0	0	0	0	0
3/1	DMBA (50 nmol/0.1 ml acetone)	16	0.1	2.9	4.3	3.0	13	80	100	60

Mice were treated with a single topical application followed 1 week later by twice weekly applications for 40 weeks.

Legends to figures

Figure 1:

Proposed pathways of metabolic activation and DNA adduct formation of 3-NBA and *N*-OH-3-ABA. See text for details. POR, cytochrome P450 oxidoreductase. R = $-\text{C}(\text{O})\text{CH}_3$ or $-\text{SO}_3\text{H}$.

Figure 2:

Levels of total DNA adducts in skin of mice treated with either 3-NBA or DMBA once (1×) or once daily for 4 days (4×) and killed 24 h or 2 weeks (2 w) after treatment. RAL (relative adduct labelling) values represent mean $\pm$ SD from two treated mice; each DNA samples was determined by two independent ^{32}P -postlabelling analyses. Insets: Autoradiographic profiles of DNA adducts obtained from mouse skin DNA treated with 3-NBA or DMBA using the ^{32}P -postlabelling assay. F=fold decrease in DNA binding in mice killed after 24 h compared to mice killed after 2 weeks.

Figure 3:

Levels of total DNA adducts in skin of mice treated with 3-NBA, *N*-OH-3-ABA or DMBA once (1×) or daily for 2 (2×) or 4 days (4×) and killed 24 h after treatment. RAL (relative adduct labelling) values represent mean $\pm$ SD from two treated mice; each DNA samples was determined by two independent ^{32}P -postlabelling analyses. Insets: Autoradiographic profiles of DNA adducts obtained from skin DNA treated once with either 3-NBA, *N*-OH-3-ABA or DMBA for 24 h using the ^{32}P -postlabelling assay. –, not determined.

Figure 4:

Levels of total DNA adducts in mice treated with 3-NBA, *N*-OH-3-ABA or DMBA (followed by twice-weekly TPA application) according to Table 1 and killed after 40 weeks. (A) DNA adduct formation in mouse skin ($n = 5$). (B) DNA adduct formation in mouse internal organs (lung, kidney, and pancreas) ($n = 4$). RAL (relative adduct labelling) values represent mean $\pm$ SD from treated mice; each DNA sample was determined by two independent ^{32}P -postlabelling analyses. Insets: Autoradiographic profiles of DNA adducts obtained from DNA treated with 3-NBA, *N*-OH-3-ABA or DMBA using the ^{32}P -postlabelling assay. ND, not detected. –, not determined.

Figure 1

Figure 2

Figure 3

Figure 4