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Polycyclic aromatic hydrocarbons derived from vehicle air pollutants in work environment and exposure to bronchus epithelial cell line (BEAS-2B) K.Savela, S. Pohjola, Finnish Institute of Occupational Health, Helsinki Finland; M.Lappi, VTT Espoo Finland Finland; L.Rantanen Fortum Oil and Gas Oy, Porvoo Finland

Abstract

Exposure to polycyclic aromatic hydrocarbons derived from vehicle air pollutants in work environment and bronchus epithelial cell line (BEAS-2B)

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Measurements of polycyclic aromatic hydrocarbons (PAHs) derived from diesel exhaust in work environment and DNA-binding of PAHs in lymphocytes and BEAS-2B cells was investigated. Exhaust particles and volatile compounds were collected on filters and polyurethane foam and PAH analysis and cell exposures were carried out using extracts of organic fraction. Air PAH concentrations and urinary metabolites were measured using HPLC and DNA adducts by ^{32}P-postlabeling assay. Workplace exposure to non-carcinogenic PAHs was low, consisting 97% of vapour phase compounds. Concentrations of 15 PAHs were 2241 and 1245 ng/m3 for exposed and 254 and 275 ng/m3 for control persons in winter and summer respectively. Seasonal variation of DNA adducts and urinary metabolites showed low internal exposure to diesel exhaust associated PAHs. After exposing BEAS-2B cells to benzo[a]pyrene, SRM 1650 diesel particulate and gasoline extracts a time- and dose-dependent adduct formation was obtained. Dose-dependent DNA adduct formation in BEAS-2B cells and PAHs analyzed in extracts correlated significantly. Biomarkers and BEAS-2B cell culture are useful tools to study diesel particulate exposure in assessing effects of carcinogenic compounds. (Supported by Finnish Academy, Fortum Oil and Gas and TEKES).

Introduction

Exhausts of urban air pollution are released straight to the breathing zone and small particles can easily penetrate into the lungs. Vehicle exhaust has been shown to contain many known carcinogenic compounds such as benzo[*a*]pyrene (B[*a*]P). Chronic exposure to particles derived from diesel and gasoline engines causes several harmful health effects, including increased lung cancer risk (IARC, 1989). Even short-term exposures to the low levels (below 100 $\mu g/m^3$) of particulate matter (PM) in air are associated with the health effects (WHO, 2000). Although the epidemiological evidence and the results from animal tests support human carcinogenicity, at least with respect to diesel exhaust emission, there is still no consensus about the mechanism of carcinogenicity. Polycyclic aromatic hyrdocarbons (PAH) are the most prominent among the genotoxic and carcinogenic agents present in polluted urban air. Genotoxic PAH compounds are known to react with DNA after their metabolic activation to form DNA adducts. This initiation step is thought to be relevant with respect to chemical carcinogenesis (Lawley, 1989; Miller, 1970). Furthermore, DNA adducts are valuable biomarkers reflecting the internal dose and exposure to PAH and nitro-PAH derived from particles. DNA adducts has been measured from test animals and from

cultured human cells and human lymhocytes after their exposure to diesel particulate or urban air extracts. (Hemminki *et al.*, 1994; Kyrtopoulos *et al.*, 2001; Nielsen *et al.*, 1996; Phillips *et al.*, 1995; Schoket *et al*., 1999).

Conclusions

Cell culture studies

- Extracts of diesel particulate were more genotoxic than those of gasoline
- PAHs measured in extracts were in good accordance with DNA adduct levels
- No large differences between gasoline and diesel fuels when calculated on particulate basis (Add/mg PM)
- When calculated as Add/mg PM/km, reformulated and standard diesel fuels formed about 10- and 30-fold more DNA adducts than gasoline

Human occupational study

- Significantly higher adduct levels among exposed than in control persons in winter ($p<0.001$) and in summer ($p<0.05$)
- Good correlation ($R=0.61$, $n=43$, $p<0.001$) between mean adduct levels and air PAHs in winter
- Low levels of particle-derived PAH exposure in work places were detected

References

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Exposure to vehicle derived diesel & gasoline exhaust – Cell culture and human studies

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External exposure

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Metabolic activation

↓

DNA adduct formation

- Indicative of carcinogenicity
- Inform the biologically effective dose
- Biomarker of exposure to PAH and nitro-PAH

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Genotoxic effects in animal and human cells

Cell culture studies

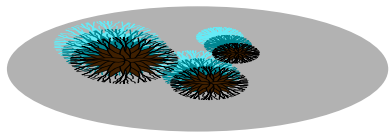
DNA damage of diesel and gasoline-derived PAHs in human bronchial epithelial cell line (BEAS-2B)

Env. Mol. Mutagenesis, 2003, 42:26-36
Mutagenesis 2003, in press

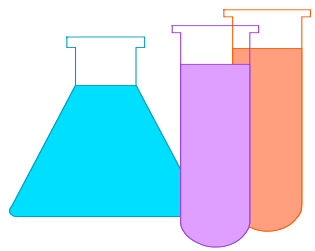
Study design

⇨ **Emission test**
-Passenger car

⇨ **Particulate sampling**
-Teflon filters and PUF



⇨ **Particulate analysis**
-GC/MS analysis of PAHs

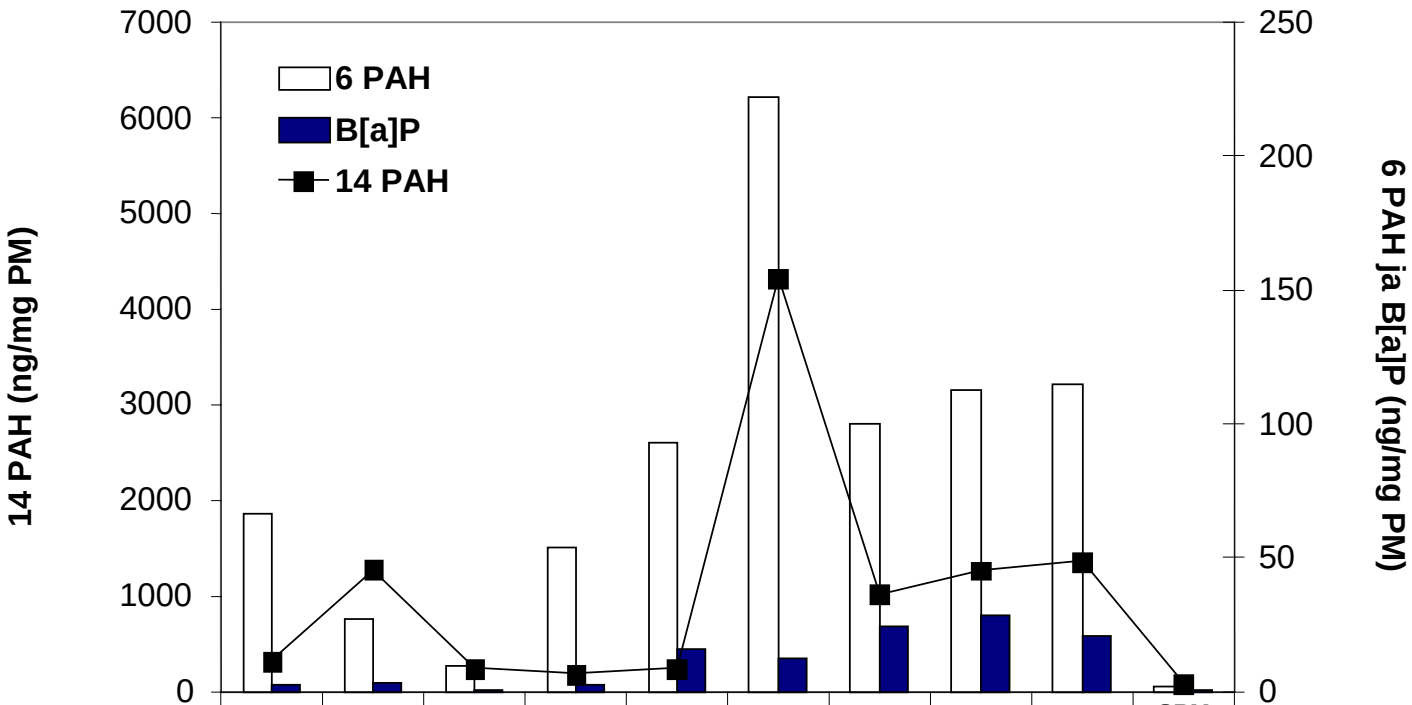


⇨ **Exposure**
-Human cell culture & CT DNA

⇨ **DNA adduct test**

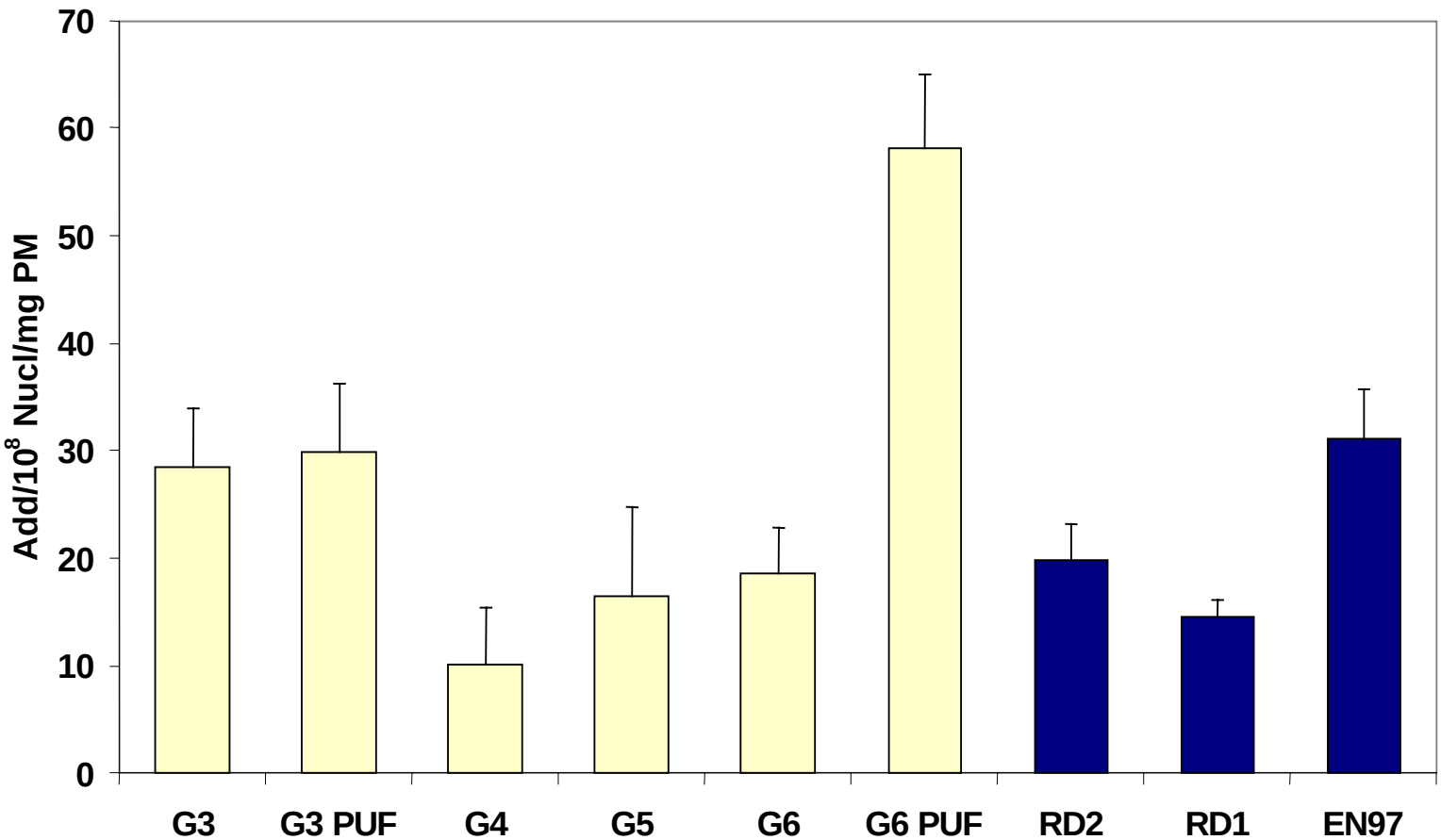
^{32}P-Postlabeling

PAHs analyzed in gasoline and diesel extracts

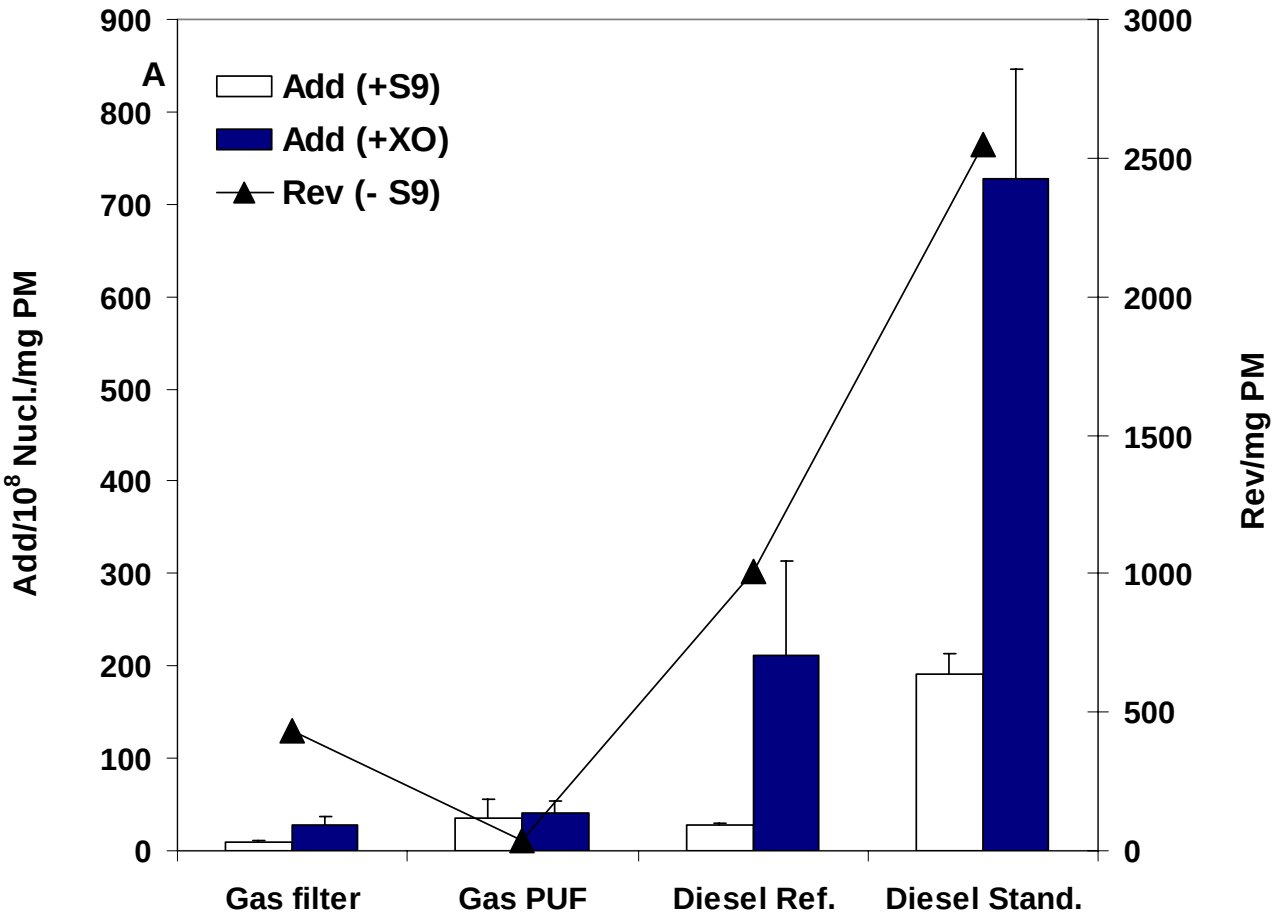


	G3	G3-PUF	G4	G5	G6	G6-PUF	RD2	RD1	EN97	SRM 1650
6 PAH	66.3	27.3	9.16	53.9	93.0	222	99.6	112	114	1.50
B[a]P	2.24	3.11	0.36	2.80	15.9	12.4	24.5	28.3	20.4	0.14
14 PAH	315.6	1277	241.6	181.0	237.3	4322	1015	1266	1356	79.8

DNA adducts formed by gasoline and diesel extracts in BEAS-2B



DNA adducts and mutagenicity formed by PAHs of diesel and gasoline particulate exhaust



Conclusion of cell studies

⇨ **No large differences between fuels when calculated on particulate basis (Add/mg PM)**

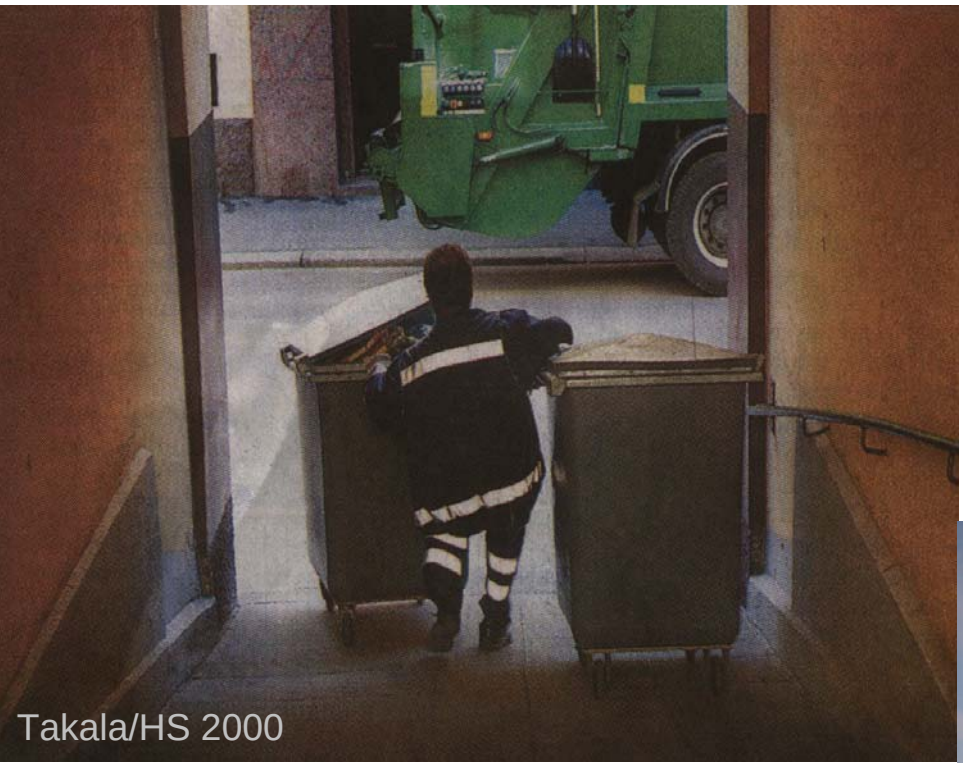
⇨ **Reformulated and standard diesel fuels formed about 10- and 30-fold more DNA adducts (Add/mg PM/km) than gasoline**

⇨ **Genotoxicity of diesel fuel based on higher particulate compared to gasoline**

Occupational exposure to diesel exhaust in bus garage and waste collection work

Bus garage

22 workers



Waste Collection

12 truck drivers

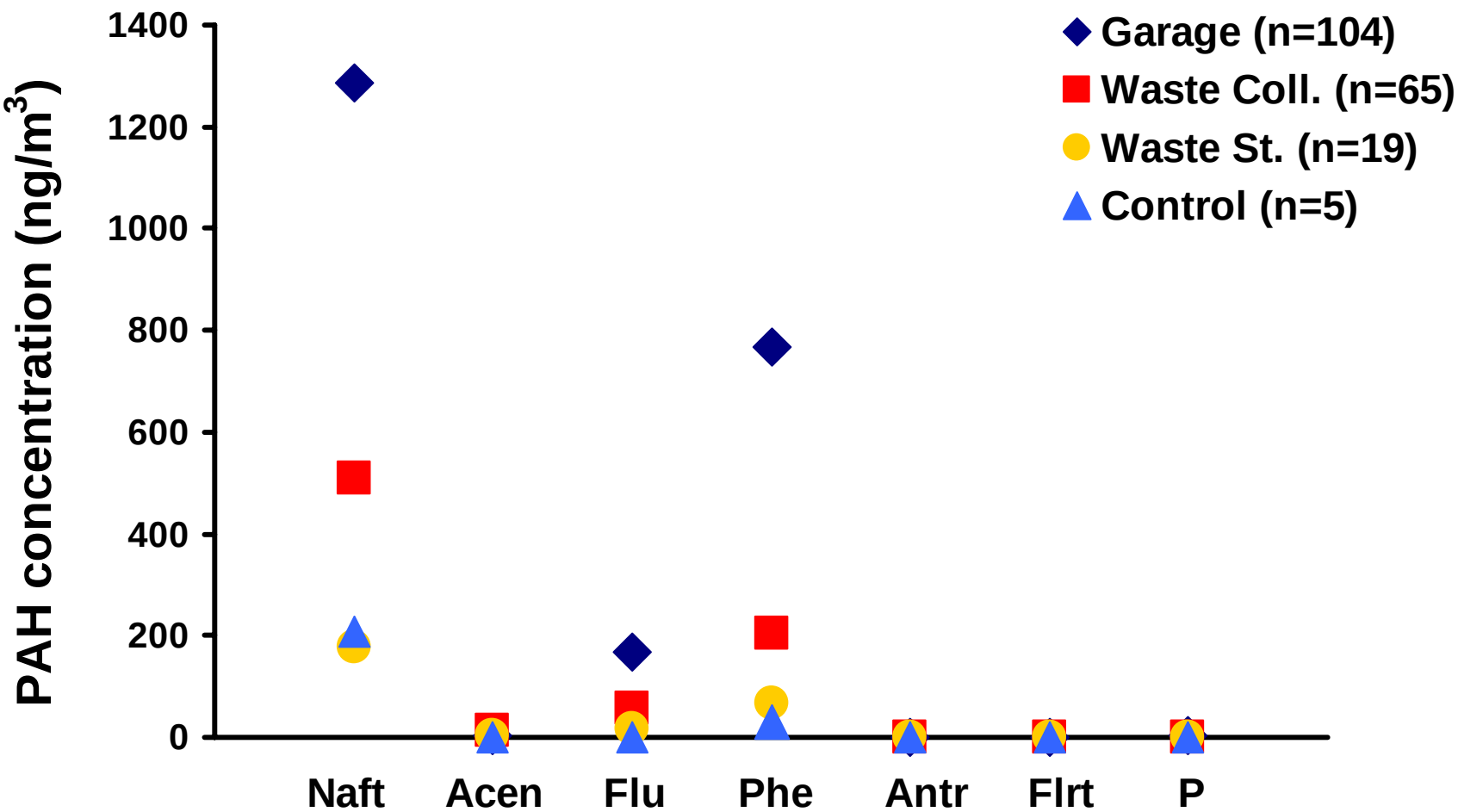
Waste Handling Centre

9 workers

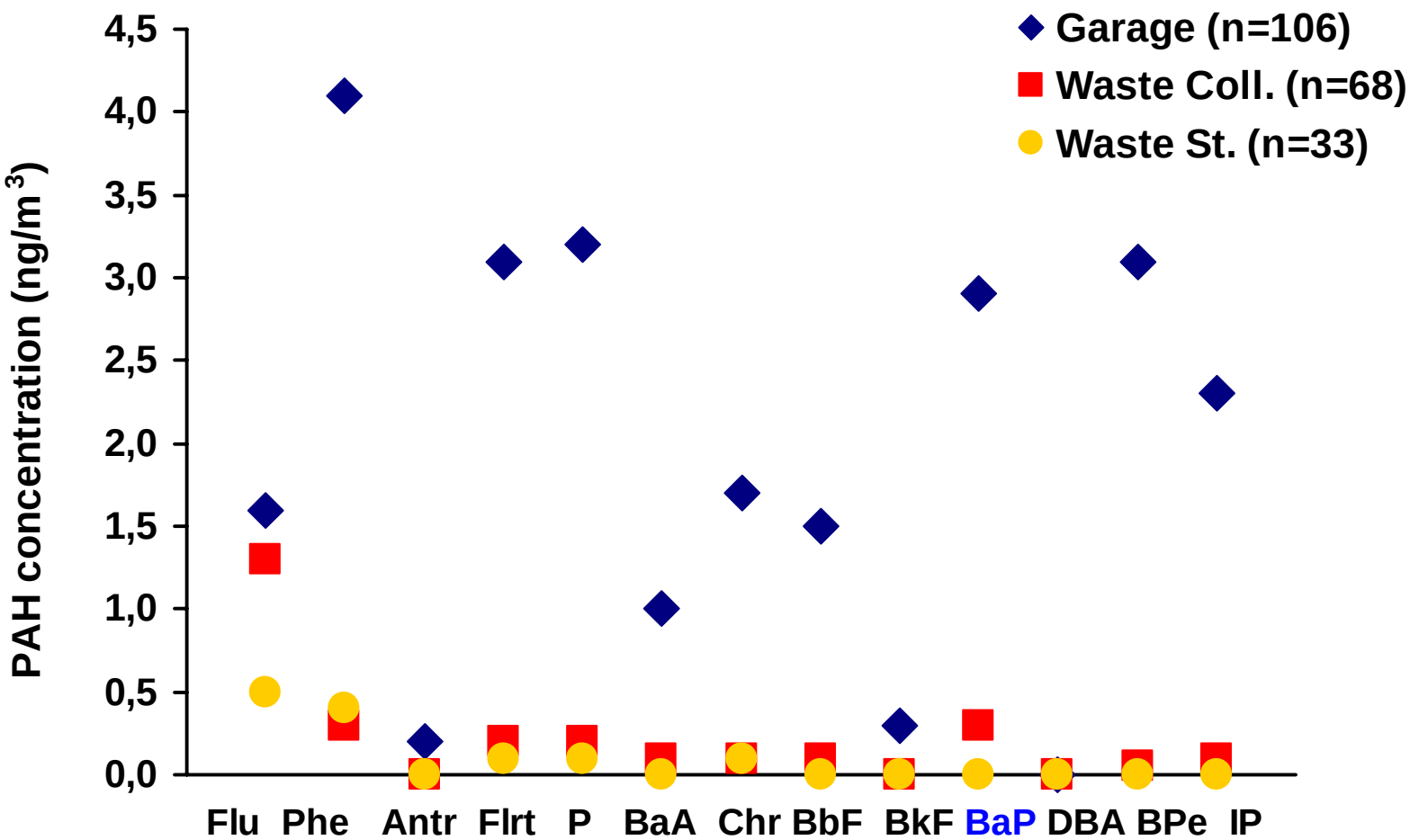
Analysis of air samples

- ***Particulate*** **on PTFE filters and PAH-compounds extracted with cyclohexane**
- ***Volatile*** **PAH extracted from XAD-2 adsorbents with acetonitrile**
- **Analysis by HPLC/FL**

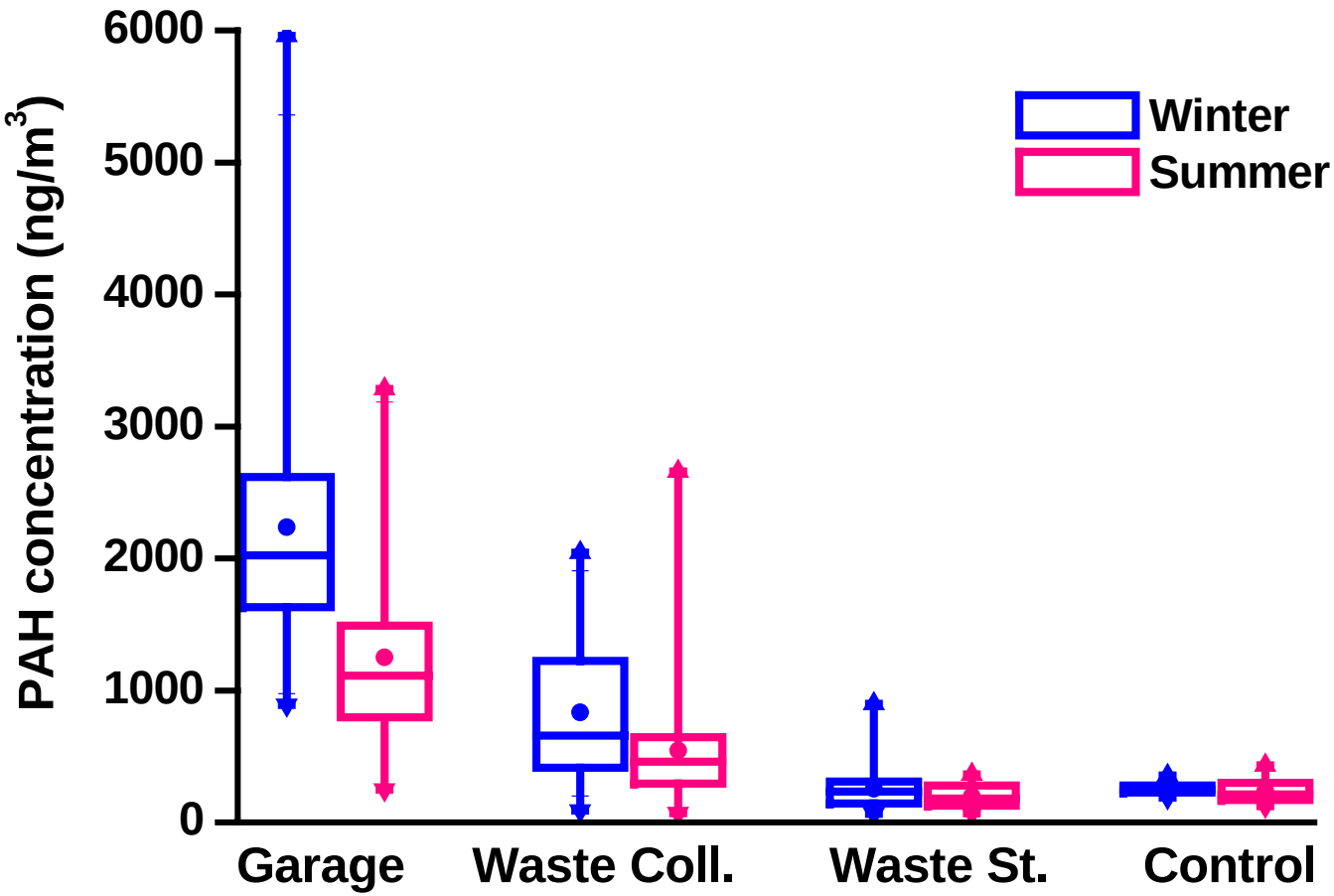
Volatile PAH compounds *in winter*



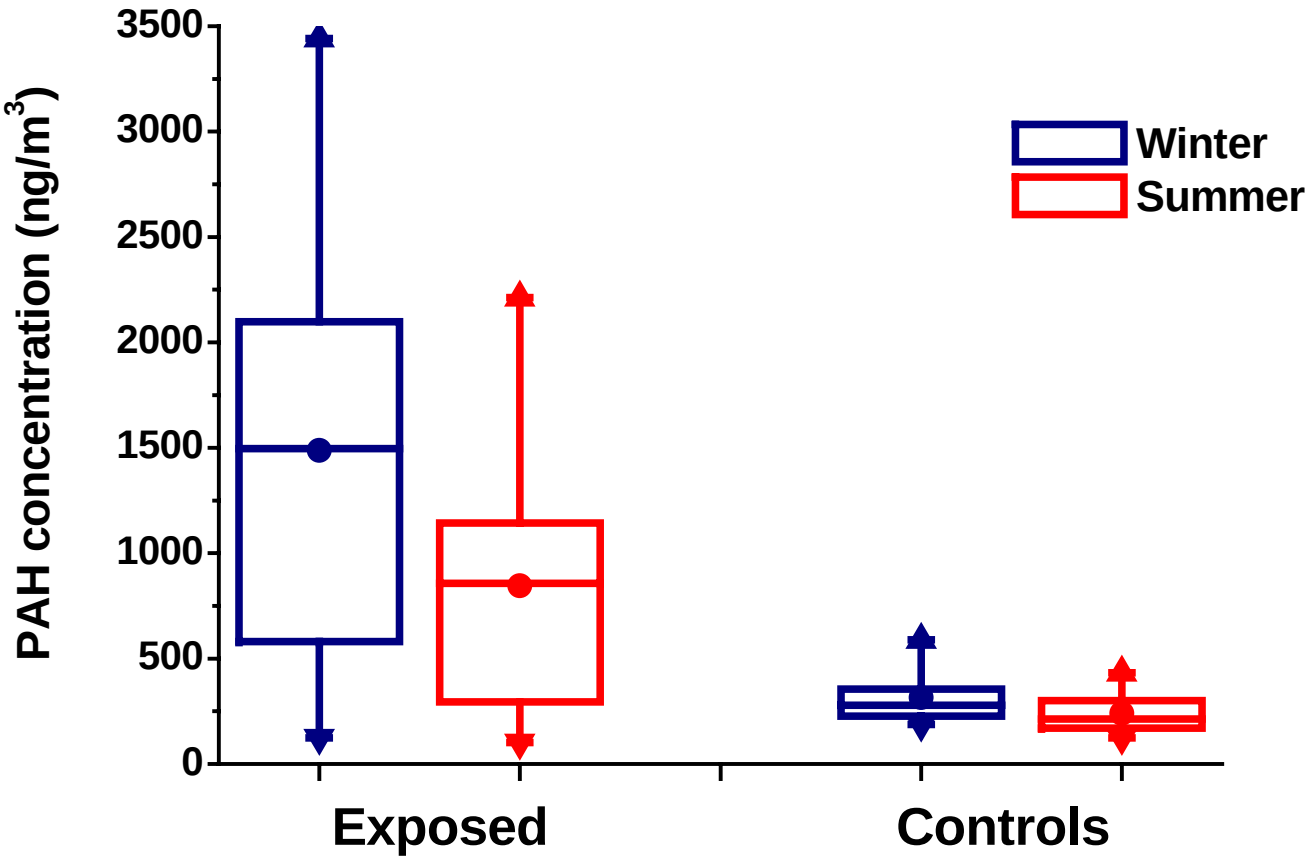
Particulate PAHs in *winter*



Total PAHs in exposure groups



Total PAHs in *winter* and *summer*



p=0.02, p=0.001

Summary of air PAH measurements

- ⇨ **Volatile PAHs > 97 %**
- ⇨ **PAHs significantly higher in exposed than in control group**
- ⇨ **Two-times higher PAH levels in winter than in summer**

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JEM 4(5)2003

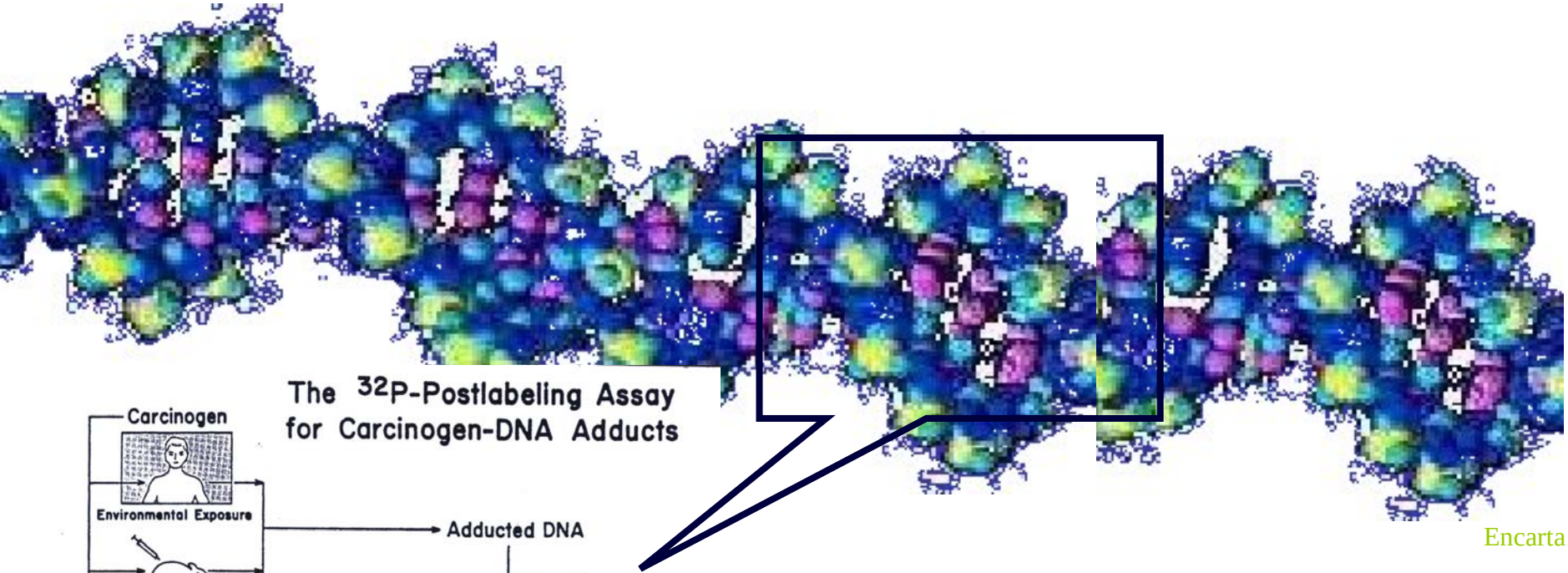
Biomonitoring samples

- ***Urine samples 3 x 2***

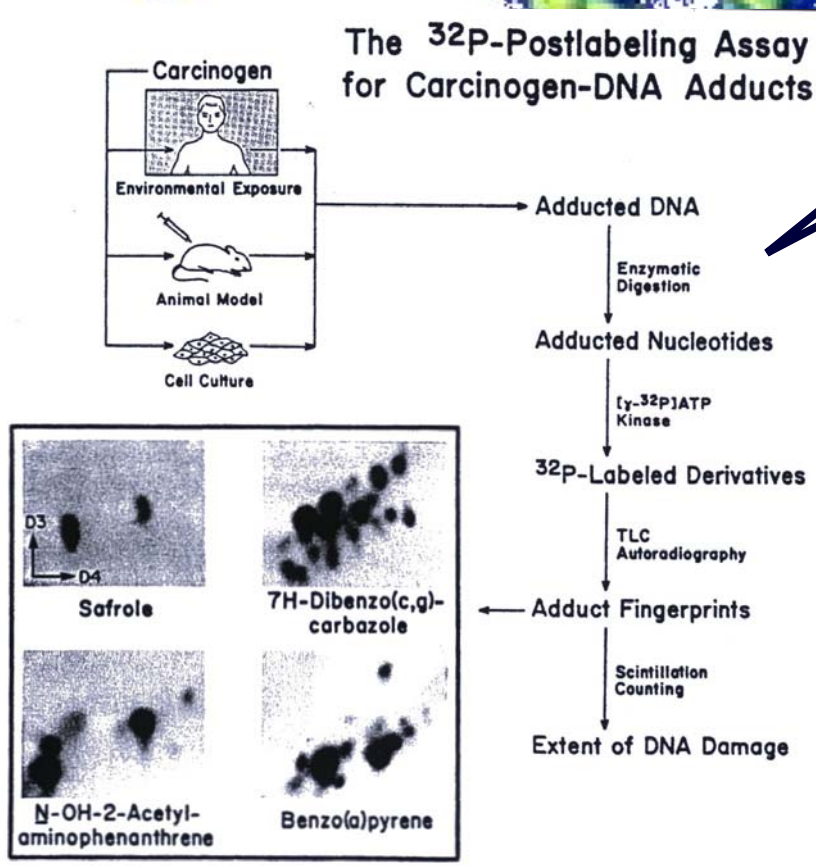
  One pre- and two post-shift samples in winter and summer

- ***Blood samples 1 x 2***

  One blood sample after sampling period in winter and summer



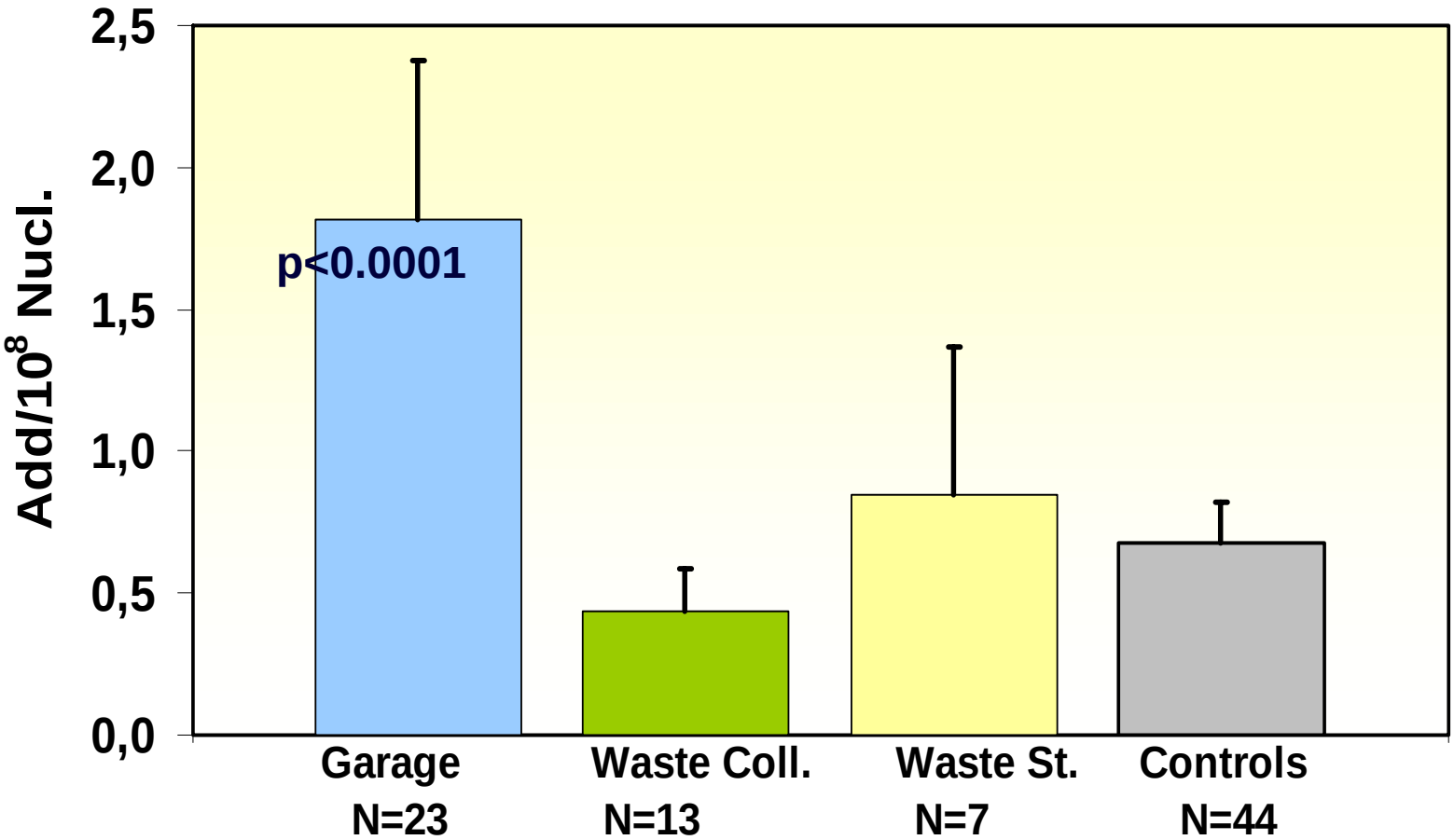
Encarta



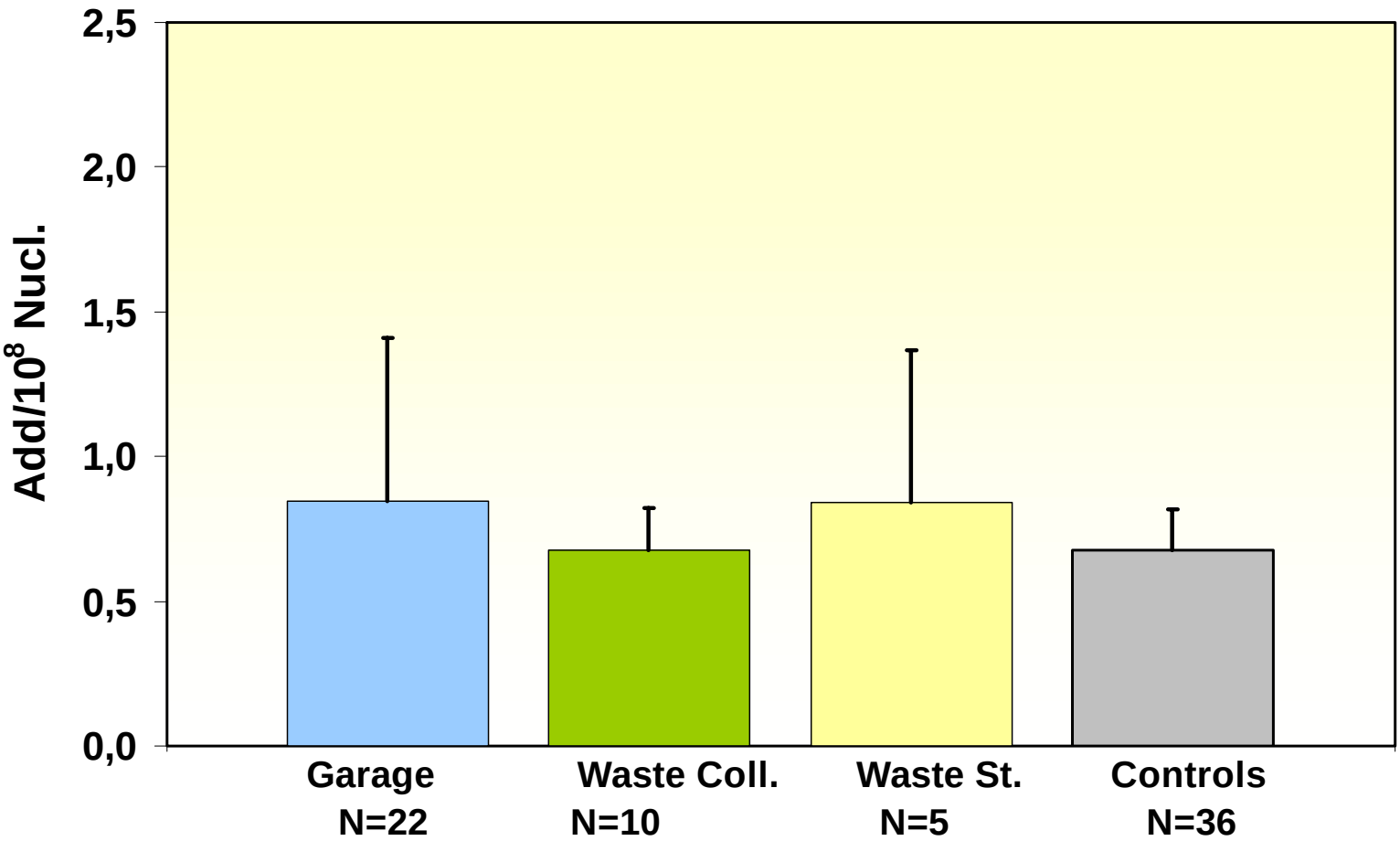
Detection of DNA adducts by ^{32}P-postlabeling assay

Cancer Research 1990

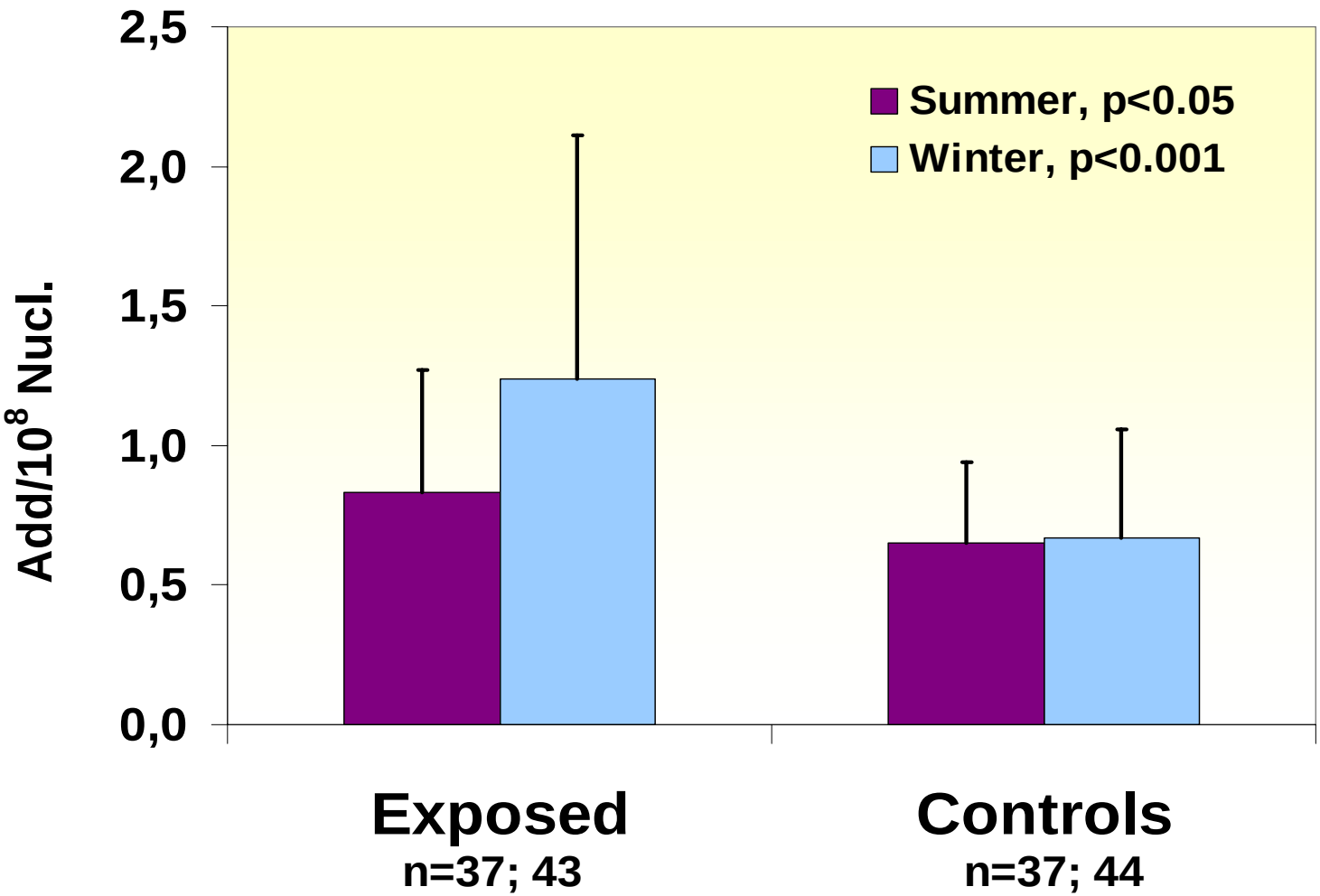
DNA adduct levels *in winter*



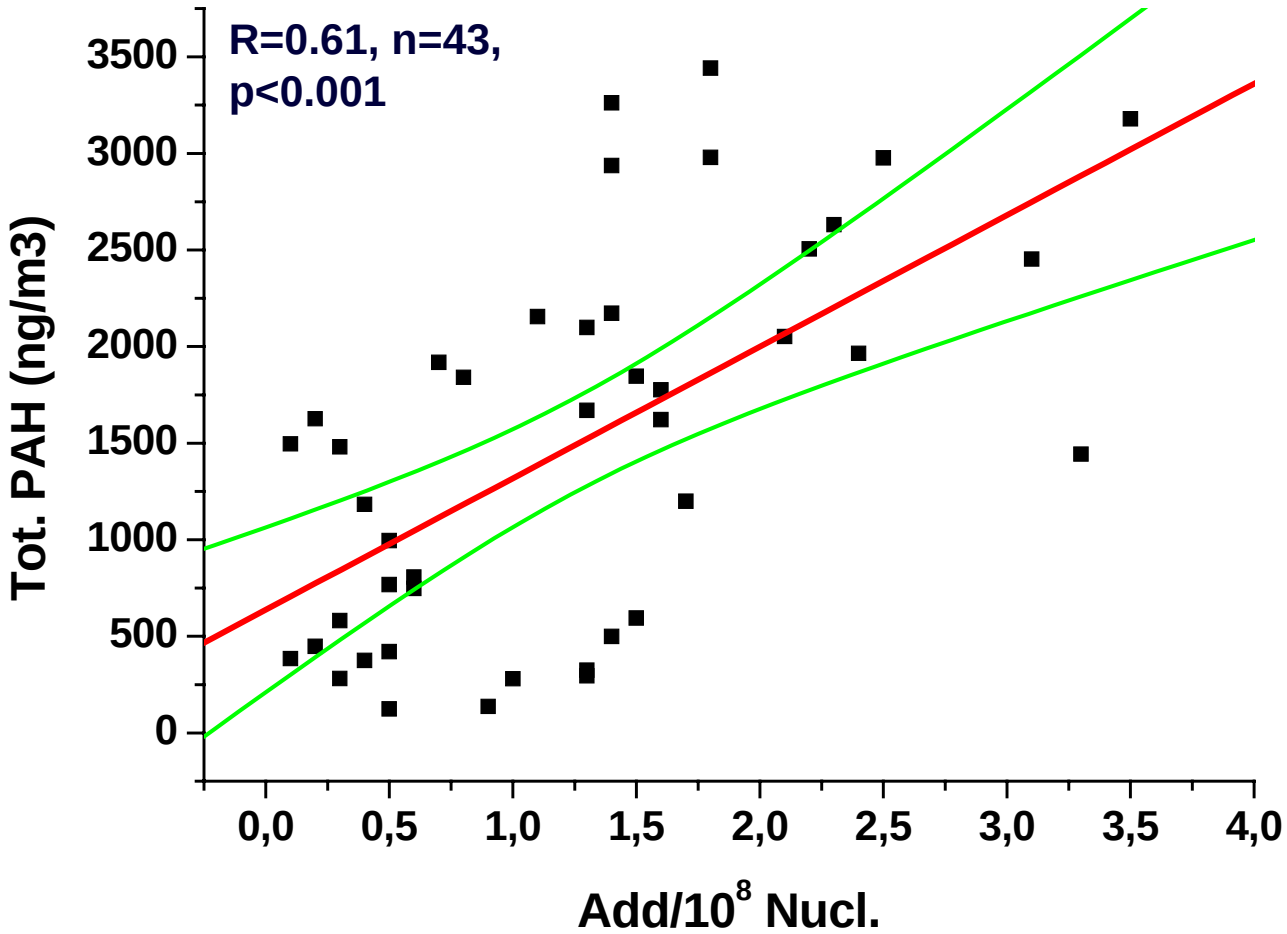
DNA adduct levels *in summer*



Tot mean DNA adduct levels



Correlation of tot DNA adducts and air PAHs *in winter*



Summary of DNA adduct results

- ⇨ **Higher DNA adduct levels in winter than in summer**
- ⇨ **Tot adduct levels of exposed significantly higher than in control persons**
- ⇨ **Good correlation ($R=0.61$, $n=43$, $p<0.001$) between tot adducts and air PAHs in winter**

Conclusions

⇨ **Extracts of diesel particulates were more genotoxic than gasoline**

⇨ **PAHs measured in extracts and air samples were in good accordance with DNA adduct levels**

⇨ **DNA adducts were 2-fold higher in exposed than control group in winter**

⇨ **Low levels of particle-derived PAHs in work places were detected**

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