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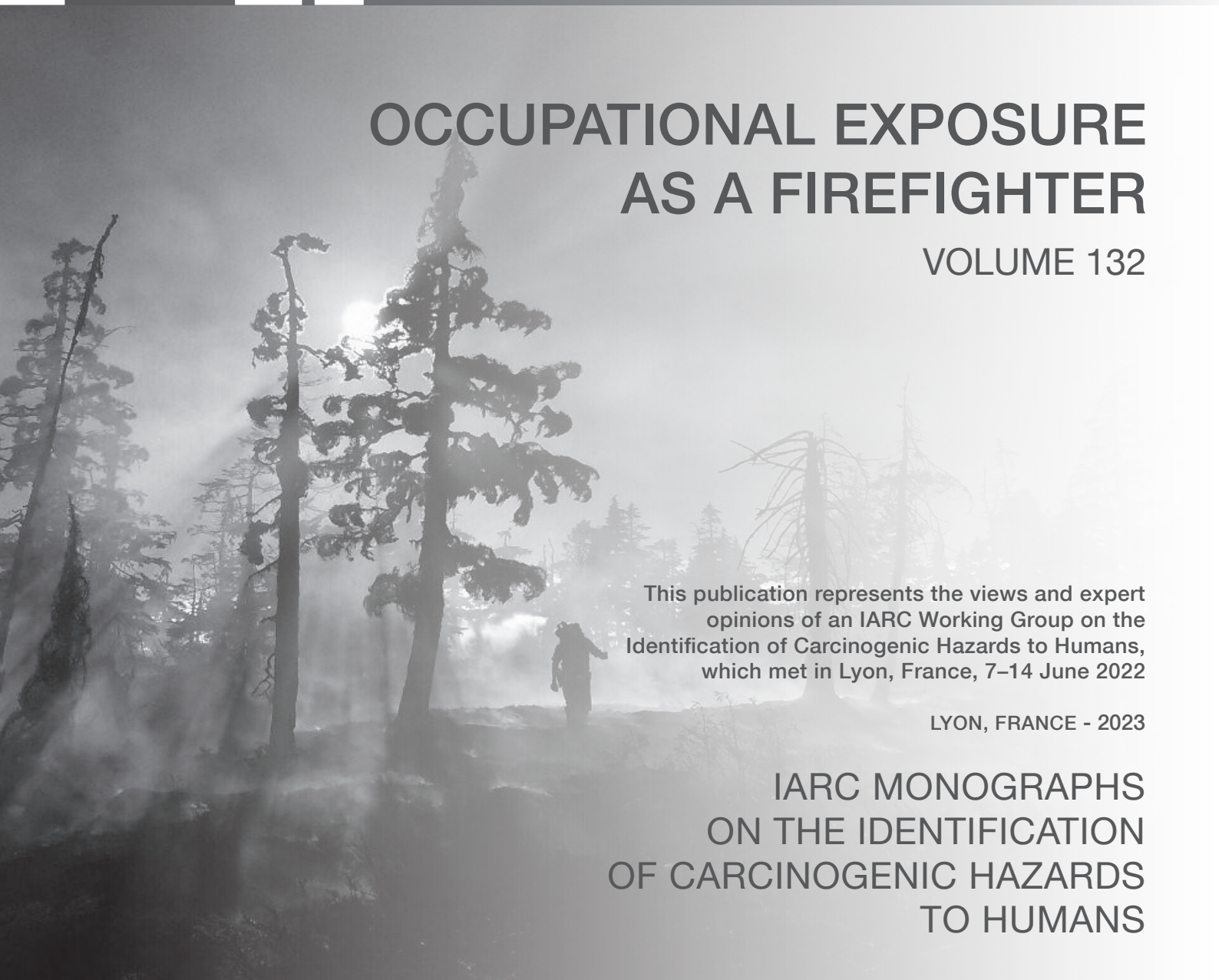

OCCUPATIONAL EXPOSURE AS A FIREFIGHTER

VOLUME 132

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IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

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4. MECHANISTIC EVIDENCE

Overview of mechanisms for carcinogens to which firefighters are exposed

Firefighters are exposed to a heterogeneous mixture of chemicals released from fires and non-fire environments. Exposure depends not only on the fuel involved and the fire conditions but also on the firefighting roles and activities being undertaken.

There is evidence that firefighters are regularly exposed to several airborne chemical agents, primarily combustion products released from fires, motor exhaust, and emissions from other activities (e.g. vehicle accidents, hazardous material releases, building collapses, and other non-emergency events) (see Section 1.2 and Section 1.3.1). Firefighters are exposed via inhalation and dermal contact to asbestos, particulate matter (PM) (coarse, fine, and ultrafine), PM-bound metals and organic compounds, airborne volatile organic compounds (VOCs) and semi-volatile organic compounds (sVOCs), flame retardants, per- and polyfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), etc. (as reported in Sections 1.4.1–1.4.4 and 1.5.1). Biomonitoring assays have also demonstrated the presence of chemical agents and/or their main metabolites on the skin and in biological fluids (e.g. urine, blood, exhaled breath) of firefighters after occupational exposure (see Section 1.4.5 and Section 1.5.1(i)). In addition, firefighters operate under conditions of extreme heat, stress, and dehydration, undertaking physical activity and night shift work.

Several of the above agents have been evaluated previously by the *IARC Monographs* programme and classified as *carcinogenic to humans* (IARC Group 1) or *probably carcinogenic to humans* (IARC Group 2A) (see Table 1.1). Their carcinogenic mechanisms as described by IARC are illustrated here. For example, asbestos, for which the primary source of exposure is structure fires or building collapse, exhibits several key characteristics of carcinogens (Smith et al., 2016) in in vitro studies; specifically, "is genotoxic"; "induces oxidative stress"; "induces chronic inflammation"; and "alters cell proliferation, cell death, or nutrient supply" (IARC, 2012a). A positive association between employment as a firefighter and mesothelioma has been observed (see Section 2.9.5).

Firefighters are exposed to PM, and the PM in outdoor air pollution has been classified as *carcinogenic to humans* (IARC Group 1). Most PM in outdoor air is a product of combustion emissions (DeMarini & Linak, 2022); as much as 25–50% of $PM_{2.5}$ (particulate matter with a diameter of 2.5 μm or less) in outdoor air in the USA originates from wildland fires (Burke et al., 2021). PM exhibits several key characteristics of

carcinogens, including "is genotoxic", "induces oxidative stress", and "induces chronic inflammation". There is strong mechanistic evidence for the genotoxicity of PM in humans (IARC, 2016).

Many known or probable human carcinogens are present in PM and are released from fires. Prominent among these are polycyclic aromatic hydrocarbons (PAHs), which have been identified in numerous exposure studies of firefighters (Section 1.4). PAHs are the chemical class most highly correlated ($r \approx 1.0$) with the mutagenicity of PM from combustion emissions (DeMarini & Linak, 2022). There is mechanistic evidence that the model PAH, benzo[*a*]pyrene (B[*a*]P), is carcinogenic to humans, exhibiting the key characteristics of carcinogens "is electrophilic or can be metabolically activated to an electrophile", "is genotoxic", "induces oxidative stress", "induces chronic inflammation", "is immunosuppressive", and "modulates receptor-mediated effects" (IARC, 2010). The only available studies were in humans exposed to mixtures of PAHs; there were no studies on exposure to B[*a*]P only. However, the finding of B[*a*]P diol epoxide–DNA adducts in humans exposed to mixtures of PAHs, together with extensive studies showing the genotoxicity of B[*a*]P in experimental systems, provided consistent and coherent mechanistic evidence for the genotoxicity of B[*a*]P in humans (IARC, 2010, 2012b).

There is mechanistic evidence, primarily electrophilicity and genotoxicity, for the carcinogenicity (IARC Group 1) of occupational exposure to complex mixtures composed predominantly of PAHs, including those encountered during coal gasification, coke production, coal-tar distillation, chimney sweeping, paving and roofing with coal-tar pitch, and aluminium production (IARC, 2010, 2012b), as well as in diesel exhaust (IARC, 2013). There is also mechanistic evidence, primarily regarding genotoxicity and electrophilicity, for the probable carcinogenicity (IARC Group 2A) of cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,l*]pyrene, and creosotes (IARC, 2010).

Exposure studies have also shown that municipal and wildland firefighters can be exposed to acrolein (IARC Group 2A), which exhibits a variety of key characteristics of carcinogens, including "is electrophilic or can be metabolically activated to an electrophile", "is genotoxic", "alters DNA repair or causes genomic instability", and "induces oxidative stress", "is immunosuppressive", "induces chronic inflammation", and "alters cell proliferation, cell death, or nutrient supply" (IARC, 2021).

Firefighters are also exposed to carcinogenic agents classified in IARC Group 1 (Table 1.1), such as benzene (IARC, 2012b, 2018) and formaldehyde (IARC, 2006, 2012b). Both compounds exhibit the key characteristics of carcinogens "is electrophilic or can be metabolically activated to an electrophile", and "is genotoxic"; in addition, benzene also exhibits the key characteristics "alters cell proliferation, cell death, or nutrient supply", "is immunosuppressive", and "modulates receptor-mediated effects". There is strong mechanistic evidence for the genotoxicity of benzene in humans, and there is moderate mechanistic evidence for the genotoxicity of formaldehyde in humans.

Other agents to which firefighters are exposed are styrene and its related metabolite, styrene-7,8-oxide, which are classified as *probably carcinogenic to humans* (IARC Group 2A). These compounds exhibit many key characteristics of carcinogens, including "is electrophilic or can be metabolically activated to an electrophile", "is genotoxic", "alters DNA repair or causes genomic instability", "alters cell proliferation, cell death, or nutrient supply", and "modulates receptor-mediated effects" (IARC, 2019). There is strong mechanistic evidence that both styrene and styrene-7,8-oxide are genotoxic, and this mechanism can also operate in humans.

Firefighters are also exposed to fire effluents such as polychlorinated dibenzo-*para*-dioxins (PCDDs, also called dioxins) and PCBs that are released in fires only when halogen-containing fuel is present (polyvinyl chloride cables, flame retardants, etc.) (see Section 1.3.1 for further details on their release from fires). 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (2,3,7,8-TCDD) is *carcinogenic to humans* (IARC Group 1) (IARC, 1997, 2012b; Table 1.1). It exhibits several key characteristics of carcinogens, including "induces oxidative stress" and "is immunosuppressive"; and there is strong mechanistic evidence in humans for "modulates receptor-mediated effects", and "alters cell proliferation, cell death, or nutrient supply" (IARC, 1997, 2012b). Several PCB congeners (IARC Group 1) exhibit the key characteristics of carcinogens "is electrophilic or can be activated to an electrophile", "is genotoxic", and "modulates receptor-mediated effects" (IARC, 2015). There is strong mechanistic support for the carcinogenicity of dioxins: receptor-mediated effects involving activation of the aryl hydrocarbon receptor (AhR) activation induce cancer in mouse skin.

Firefighters can be exposed to various carcinogenic (IARC Group 1) metals, including chromium(VI), nickel, and cadmium (IARC, 2012a). These metals cause cancer by genotoxic mechanisms, and chromium(VI) and nickel also affect DNA repair.

Solar radiation, which is classified in IARC Group 1 and causes skin cancer in humans (IARC, 2012c), is also a component of occupational exposure as a firefighter. Solar radiation exhibits a variety of carcinogenic mechanisms, including genotoxicity, induction of DNA repair, and immunosuppression (IARC, 2012c).

Firefighters may undertake night shift work, previously classified as IARC Group 2A (IARC, 2020) (see Section 1.5.2(a) and Table 1.1). There is mechanistic evidence in experimental systems that night shift work exhibits key characteristics of carcinogens, such as "induces chronic inflammation", "is immunosuppressive", and "alters cell proliferation, cell death, and nutrient supply" (IARC, 2020). There is suggestive mechanistic evidence in humans that night shift work alters levels of estrogen, and there is robust evidence that it alters levels of melatonin.

Therefore, occupational exposure as a firefighter encompasses a wide range of agents, including physical, chemical, and/or behavioural human carcinogens and probable human carcinogens, which exhibit a variety of key characteristics of carcinogens (Smith et al., 2016).

4.1 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the key characteristics of carcinogens (Smith et al., 2016) encompassed by the agent "occupational exposure as a firefighter". The mechanistic studies were mainly conducted in humans, and the exposure assessments for these studies are reported in Table S1.30 (see Annex 1, Supplementary material for Section 1, online only, available from: https://publications.iarc.fr/615).

Evidence was available on whether occupational exposure as a firefighter exhibits the key characteristics "is genotoxic", "induces oxidative stress", "induces epigenetic alterations", "induces chronic inflammation", "is immunosuppressive", and "modulates receptor-mediated effects". Insufficient data were available for the evaluation of other key characteristics of carcinogens. Mechanistic studies in exposed humans are described in the following categories: (i) structure fires; (ii) wildland fires; (iii) employment as a firefighter; (iv) heat, mental, and/or physical challenge; and (v) catastrophic events. The "structure fires" and "wildland fires" categories were used for studies in which the authors specifically reported the type of fire to which the participants were exposed. The "employment

as a firefighter" category was used when it was unclear what type of fire the firefighters were exposed to or when firefighters may have been exposed to different fire types during the studied period. The "heat, mental, and/or physical challenge" category contains studies in which the studied effect was related to heat or mental and/or physical challenge. The "catastrophic events" category contains studies on firefighters who were exposed to specific agents while responding to a catastrophic event, such as a terrorist attack or chemical factory explosion. These types of exposure are unique events that are unlikely to apply to most firefighters. Not all sections contain all categories, depending on the studies available for each key characteristic of cancer. Within each section, the most informative studies are described first.

4.1.1 Is genotoxic

(a) Exposed humans

See Table 4.1 and Table S1.30 (see Annex 1, Supplementary material for Section 1, online only, available from: https://publications.iarc.fr/615).

(i) Structure fires

Only one study, in firefighters in Canada, examined genetic toxicity after on-shift exposure of all study participants to structure fires. In this study, 31 paired samples of urine collected pre (spot sample) and post (18-hour integrated sample) 24-hour shifts were obtained from 16 non-smoking male municipal firefighters over the course of 19 emergency fire suppression events. Samples were only collected for shifts during which emergency fire suppression events took place (Keir et al., 2017). The unexposed control group included 17 non-smoking male office workers, from whom 18 spot urine samples were collected. Study participants did not consume charbroiled foods and were not exposed to non-occupational combustion sources during the study period. Deconjugated urine extracts were assessed for urinary mutagenicity in bacteria, using the plate incorporation version of the Ames/*Salmonella* reverse mutation assay (*Salmonella typhimurium* strain YG1041 + S9, 9000 × *g* supernatant). There was a significant fold-change of 4.3 in creatinine-adjusted urinary mutagenicity in the post-fire samples compared with the pre-fire samples. There was also a significantly higher level of creatinine-adjusted urinary mutagenicity in the post-fire samples compared with the office worker controls. There was no significant difference in levels of creatinine-adjusted urinary mutagenicity between samples from the office workers and pre-fire samples (Keir et al., 2017). [The Working Group noted that this study was particularly informative because of several aspects of the study design, specifically, because confounding exposures were minimized or eliminated, all individuals participated in on-shift fire suppression events, samples were collected during a reasonable time frame for the end-point examined, and post-exposure samples were compared with pre-exposure paired samples as well as non-firefighter controls.]

DNA damage, measured by the alkaline comet assay, was assessed in peripheral blood mononuclear cells (PBMCs) collected from 12 female and 41 male non-smoking individuals undergoing a 9-month rescue-specialist educational course (Andersen et al., 2018a). Peripheral blood samples were obtained 14 days before a 3-day firefighting exercise (i.e. pre-exposure), immediately after the 3-day course (i.e. post-exposure), and 14 days post-exposure (i.e. 14-day). Firefighting exercises involved the extinction of fires started from wood fuel or from mixed fuel (i.e. wood with foam mattresses and electrical cords). There was a significant increase in DNA damage in post-exposure samples compared with 14-day samples; however, no significant difference for this end-point was found between the pre-exposure and post-exposure samples, nor when the pre-exposure and 14-day samples

Table 4.1 Genetic and related effects in exposed firefighters

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Structure fires							
Urinary mutagenicity (Ames/ Salmonella, YG1041 + S9)	Urine (creatinine-corrected)	Structural [municipal] firefighters Canada, pre/post, 31 samples collected from 16 non-smoking male municipal firefighters pre (spot sample) and post (18-h integrated sample) fire suppression events. Unexposed controls: 18 spot samples collected from 17 non-smoking male office workers. Study participants did not consume charbroiled food and were not exposed to non-occupational combustion sources throughout the study.	16 (31 paired samples, post-fire and pre-fire)	+ ($P < 0.001$)	None	Only municipal firefighter study that examined genotoxicity after on-shift exposure of all individuals to structure fire(s) Exposure assessment: appropriate personal shift PAH exposure measure; firefighting was appropriately evaluated as exposure in the pre/post design	Keir et al. (2017)
			16, 17 (31 post-fire samples from 16 firefighters and 18 control samples from 17 office workers)	+ ($P < 0.001$)			
			16, 17 (31 pre-fire samples from 16 firefighters and 18 control samples from 17 office workers)	–			

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
DNA damage (alkaline comet assay)	PBMC	Training Denmark, pre/post, 53 (12 women and 41 men) non-smoking participants undergoing a 9-month rescue specialist educational course. Firefighting exercises involved extinction of fires from wood fuel or from mixed fuel (i.e. wood with foam mattresses and electrical cords). Samples obtained 14 days before a 3-day firefighting exercise (i.e. pre-exposure), immediately after exposure (i.e. post-exposure), and 14 days post-exposure (i.e. 14-day).	53 (paired samples, post-exposure, and 14 days after)	+ ($P < 0.01$) DNA damage (DSB) frequency was found to be positively correlated with urinary 1-OHP concentration ($P < 0.001$), skin pyrene concentration ($P < 0.001$), and with skin total PAH concentration ($P < 0.001$)		Comet scoring carried out by visual classification into 5 classes rather than by digital image analysis	Andersen et al. (2018a)
			53 (paired samples, post-exposure and pre-exposure)	–		Collection window of 3 days for the post-exposure samples may have been too long to be able to detect some of the exposure-induced DNA damage, potentially resulting in a reduced signal in those samples Pre-exposure samples were collected 2 wk before exposure	
			53 (paired samples, 14-day and pre-exposure)	–			
			53 (paired samples, post-exposure and mean of pre-exposure and 14-day)	–		Pre-exposure samples were collected 2 wk before exposure. Exposure assessment: appropriate personal shift PAH and 1-OHP exposure measures; firefighting was appropriately evaluated as exposure in the pre/post design	

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Wildland fires							
Urinary mutagenicity (Ames/ Salmonella, YG1041 + S9)	Urine	Prescribed burns (wildland firefighters) USA, 2015–2018, pre/ post, 19 healthy wildland firefighters (17 men, 2 women) taking part in prescribed burn practices. Samples collected immediately before (pre-shift), immediately after (post-shift), and the morning following (next morning) their shifts. Sampling took place for both prescribed burn (burn day) and regular (non-burn day) work shifts.	19 (27 paired samples, post-shift and pre-shift, 7 burn days)	Crude urine: + ($P < 0.01$) Creatinine-corrected urine: – Cross-shift change in creatinine-corrected urinary mutagenic potency significantly associated with length of smoke exposure ($P = 0.01$)	Burn day participants only	Burn day average shift length, 4.98 ± 1.34 h Number of days between studied shift and previous shift not reported (applies to all entries for this study) No non-firefighter controls (applies to all entries for this study) A significant negative correlation was reported between pre-shift to next-morning creatinine-adjusted urinary mutagenic potency and the concentration of black carbon (as measured using a personal sampler) in wildland fire smoke emissions during the prescribed burn ($P = 0.04$); this result suggested that personal exposure measurements may not be reflective of internal dose among exposed firefighters Exposure assessment: Appropriate personal shift $PM_{2.5}$ and black carbon exposure measures; firefighting was appropriately evaluated as exposure in the pre/post design	Wu et al. (2020a)
		Prescribed burns (wildland firefighters)	19 (27 paired samples, next morning and pre-shift, 7 burn days)	Crude urine: – Creatinine-corrected urine: –	Burn day participants only	Burn day average shift length, 4.98 ± 1.34 h	

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Urinary mutagenicity (Ames/ Salmonella, YG1041 + S9) (cont.)		None (wildland firefighters)	19 (14 paired samples, post-shift and pre-shift, 3 non-burn days)	Crude urine: – Creatinine-corrected urine: –	Non-burn day participants only	On non-burn days, firefighters worked at the forest office, with few exceptions Non-burn day shift length not reported	Wu et al. (2020a) (cont.)
		None (wildland firefighters)	19 (10 paired samples, next morning and pre-shift, 3 non-burn days)	Crude urine: – Creatinine-corrected urine: –	Non-burn day participants only	On non-burn days firefighters worked at the forest office with few exceptions Non-burn day shift length not reported	
Urinary mutagenicity (Ames/ Salmonella, YG1041 + S9)	Urine	Prescribed burns (wildland firefighters) USA, 2015, pre/post, 12 healthy non-smoking wildland firefighters (9 men, 3 women) taking part in prescribed burn practices. Samples collected immediately before (pre-shift), immediately after (post-shift) and the morning following (next morning) their shifts. Sampling took place for both prescribed burn (burn day) and regular (non-burn day) work shifts.	12 (48 paired samples, post-shift and pre-shift, 7 burn days)	Crude urine: – Creatinine-corrected urine: – Mean cross-shift changes in urinary mutagenicity were routinely higher for burn day samples, in comparison with non-burn day samples Significant positive associations were observed between the cross-shift change in creatinine-corrected urinary mutagenicity for all study participants and the concentration of urinary MDA ($P = 0.0010$), as well as with urinary 1-OHP ($P = 0.0001$)	Burn day participants only	Pilot study, had small sample size Number of days between last prescribed burn shift and burn day work shift ranged from 1 to > 30; no non-firefighter controls No respiratory protection Exposure assessment: Appropriate personal shift, light-absorbing carbon of $PM_{2.5}$ measured as a surrogate for black carbon, and 1-OHP exposure measures; firefighting was appropriately evaluated as exposure in the pre/post design	Adetona et al. (2019)

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Urinary mutagenicity (Ames/ Salmonella, YG1041 + S9) (cont.)	Urine (crude)		12 (40 paired samples, next morning and pre-shift, 7 burn days)	Crude urine: – Creatinine-corrected urine: –	Burn day participants only		Adetona et al. (2019) (cont.)
	Urine	None (wildland firefighters)	8 (19 paired samples, post-shift and pre-shift, 3 non-burn days)	Crude urine: – Creatinine-corrected urine: –	Non-burn day participants only	Pilot study, had small sample size Number of days between last prescribed burn shift and non-burn day work shift ranged from 3 to 30; no non-firefighter controls On non-burn days, participants reported occupational exposures to vehicle exhaust, diesel, dust, or possible exposures to smoke from nearby smouldering fires	
	Urine		8 (16 paired samples, next morning and pre-shift, 3 non-burn days)	Crude urine: – Creatinine-corrected urine: –	Non-burn day participants only		
DNA damage (alkaline comet assay)	PBMC	None (wildland firefighters) Portugal, cross-sectional, 60 volunteer wildland firefighters with ≥ 1 yr experience and 63 office-worker controls matched on age, gender, and smoking habits.	60, 63	+ ($P < 0.05$)	No significant effects of gender or smoking habits	No specific exposure event Includes current smokers Exposure assessed on the basis of duration of firefighting PPE use unknown; variable was excluded due to small number of responses to this question on questionnaire; PPE misuse is common while fighting wildland forest fires Exposure assessment: no information on specific exposures	Abreu et al. (2017)

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
DNA damage (alkaline comet assay) (cont.)		The study population was stratified into 3 age groups: < 29 yr, 29–38 yr, > 38 yr	20, 19 (< 29 yr), 20, 24 (> 38 yr)	–	No significant effects of gender or smoking habits		Abreu et al. (2017) (cont.)
			20, 20 (29–38 yr)	+ ($P < 0.05$) For exposed volunteer firefighters, level of DNA damage was higher in those aged 29–38 vs < 29 yr ($P < 0.05$)			
		Portugal, cross-sectional, 10 female and 50 male volunteer wildland firefighters with ≥ 1 yr experience and 10 female and 53 male office-worker controls matched on age, gender, and smoking habits.	10, 10 (women), 50, 53 (men)	–			
		Portugal, cross-sectional, 16 smoker and 44 non-smoker volunteer wildland firefighters with ≥ 1 yr experience and 16 smoker and 47 non-smoker office-worker controls matched on age, gender, and smoking habits.	16, 16 (smoker), 44, 47 (non-smoker)	–			

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
DNA adducts (PAH–DNA adducts, ELISA)	PWBC	Wildland USA, 1988, repeated measurements, 37 male and 10 female non-smoking wildland firefighters. Samples obtained 8 wk apart, during early and late forest fire season.	47 (paired samples, late and early forest fire season)	– Additionally, PAH–DNA adduct levels were not associated with cumulative hours of recent firefighting activity; results unaffected when controlling for frequency of charbroiled food consumption. In a follow-up study (Rothman et al., 1995), the impacts of the *GSTM1*-null genotype and *CYP1A1* exon 7 polymorphisms on PAH–DNA adduct levels were investigated; no significant results were found. Early vs late time-points were not compared (i.e. no exposed vs control) within the genotype analysis. There was no association between the PAH–DNA adduct levels and the cumulative hrs of recent firefighting activity in *GSTM1*$^{-/-}$ participants or in those without this genotype	Measures of previous firefighting activity	Did not control for consumption of charbroiled food in late vs early season comparison No non-firefighter controls; for early and late time-points, respectively, there were means of 16 ± 3.15 h and 97.38 ± 15.26 h of self-reported firefighting activity in the 4 wk preceding blood collection Paired samples from same individuals were treated as independent; authors justified this choice by demonstrating that the correlation between repeat adduct measurements was low Exposure assessment: good approach to semiquantitative exposure estimation from questionnaire (prospectively collected activity diary – may be affected by degree of completion)	Rothman et al. (1993)

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
DNA damage (alkaline comet assay)	PBMC	Wildland Portugal, cross-sectional, 93 non-smoking control firefighters, 48 non-smoking exposed firefighters, and 30 smoking exposed firefighters. Exposed firefighters participated in fire suppression activities within 48 h of sampling. Participants excluded if recently consumed grilled, barbecued, or smoked foods.	48 (non-smoking exposed), 93 (non-smoking control) 30 (smoking exposed), 93 (non-smoking control)	– –		No non-firefighter controls or pre/post sampling of the same individuals. All 3 groups reported long-term (i.e. median, > 10 yr) exposure to forest fire emissions Collection window of 48 h may have been too long to be able to detect DNA damage Exposure assessment: firefighting status used for comparison with controls and biomonitoring data used for correlation analysis limited because of only post-exposure collection	Oliveira et al. (2020)
Employment as a firefighter							
Micronucleus frequency	Exfoliated buccal epithelial cells	None (municipal firefighters) India, cross-sectional, 47 male firefighters with ≥ 10 yr service and 40 male office worker controls matched on age, ethnicity, food habit, smoking status, alcohol consumption, nutritional status, and the extent of indoor air pollution in their homes.	47, 40 27 (firefighters served ≥ 20 yr), 20 (firefighters served ≥ 10 to < 20 yr)	+ ($P < 0.01$) + ($P < 0.05$)	Stratified by duration of service	No specific exposure event Exposure assessment: qualitative exposure assignment based apparently on self-report; employment status probably adequate for comparisons made	Ray et al. (2005)

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
DNA adducts (PAH–DNA adducts, ELISA)	PBL	None (municipal firefighters) USA, cross-sectional, 43 male municipal firefighters and 40 male controls matched on age and smoking status.	43, 38	–	Consumption of charbroiled foods, smoking, alcohol intake, race	No specific exposure event; exposure based on history of firefighting activities Study included current smokers and 7 controls had history of occupational exposure to mutagens PPE use was variable Exposure assessment: adequate for primary hypothesis of higher biomarker (DNA damage) levels in firefighters vs controls	Liou et al. (1989)
			37, 29	+ (OR, 3.36; 95% CI, 1.08–10.5)	Consumption of charbroiled foods plus race as White		
			6, 9	–	Consumption of charbroiled foods plus race as non-White	Small sample size	
Sister-chromatid exchange	PBL	None (municipal firefighters)	42, 38	–	Race, history of viral infections, frequency of exposure, PPE use, duration of employment	No specific exposure event; exposure based on history of firefighting activities Study included current smokers and 7 controls had history of occupational exposure to mutagens PPE use was variable Exposure assessment: adequate for primary hypothesis of higher biomarker (DNA damage) levels in firefighters vs controls	

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Sister-chromatid exchange	PBL	None (municipal firefighters) Japan, 1998, cross-sectional, male municipal firefighter controls, non-smoking male general population controls matched on age; 2 control populations from the Tokyo sarin disaster study (both not exposed to sarin).	9 (non-smoker firefighter control), 11 (non-smoker general control)	+ ($P < 0.01$)	Non-smoker	No specific exposure event Age not well matched between groups (47.0 ± 2.6 vs 41.5 ± 2.8)	Li et al. (2004)
Miscarriage	NA	None (municipal and wildland firefighters) USA, 2017–2019, cross-sectional, self-reported most recent pregnancy outcome in 1041 female firefighters and 7482 female nurses.	1041, 7482	+ (aSPR, 2.33; 95% CI, 1.96–2.75)	Age	Indirect assessment of genotoxicity	Jung et al. (2021a)
DNA damage (alkaline comet assay)	PBMC	Structural or none (municipal firefighters) Denmark, pre/post, 22 male firefighters, samples collected before and after a 24-h shift.	22 (paired samples, after and before)	–		Firefighters had 3 days off between work shifts Only 14/22 firefighters reported participation in firefighting activities and/or exposure to smoke during their shift Study included current smokers Comet scoring carried out by manual visual classification rather than by digital image analysis Exposure assessment: Firefighting was appropriately evaluated as exposure in the pre/post design; other exposure measures apparently not used in effect analysis; some logistic difficulties	Andersen et al. (2018b)

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
DNA damage (alkaline comet assay) (cont.)			14 (paired samples, after and before)	–	Participated in fire extinction activities	Small sample size	Andersen et al. (2018b) (cont.)
			8 (paired samples, after and before)	–	Did not participate in fire extinction activities	Small sample size	

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Catastrophic events							
Somatic mutations (i.e. clonal haematopoiesis detected by deep targeted sequencing)	PWBC	WTC event USA, 2013–2015, cross-sectional, 429 WTC-exposed firefighters and 255 non-WTC-exposed firefighters	429, 255	+ (OR, 2.93; 95% CI, 1.52–5.65; $P = 0.0014$) Result also significant when restricted to firefighters with smoking information and controlling for smoking (OR, 2.78; 95% CI, 1.39–5.59; $P = 0.004$) In both the WTC-exposed and firefighter control populations, mutations were predominantly in *DNMT3A* and *TET2* (involved in DNA methylation control) and were also found in several cancer associated genes (i.e. *TP53*, *U2AF1*, *PTEN*, *TERT*) Most common COSMIC mutation signatures observed in the WTC-exposed firefighters were: (1) ageing; (2) DNA mismatch repair; (3) smoking; and (4) alkylating agents	Age, sex, race/ethnicity	No non-firefighter control group COSMIC mutational signatures were not reported for the non-WTC-exposed firefighters	Jasra et al. (2022)

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Sister-chromatid exchange	PBL	Tokyo sarin disaster (municipal firefighters) Japan, 1998, cross-sectional, male municipal firefighters exposed to sarin while responding to the Tokyo sarin attack, male municipal firefighters not exposed to sarin matched on age and smoking status, non-smoking male general population controls matched on age. Samples obtained 3 yr after exposure. Sarin exposure confirmed by serum ChE activity measured at the time of exposure.	27 (firefighter exposed), 18 (firefighter control)	+ ($P < 0.05$) A significant ($P < 0.05$) positive correlation was observed between the frequency of SCEs in PBLs and the rate of serum ChE activity decrease in the sarin-exposed firefighter group		Unique exposure with limited relevance to the hazards of typical firefighters Exposure assessment: adequate to establish exposed vs unexposed to one-time exposure to sarin and contaminants	Li et al. (2004)
			15 (smoker firefighter exposed), 9 (smoker firefighter control)	+ ($P < 0.05$)	Smoker	Age not well matched (43.0 ± 2.9 vs 38.8 ± 4.1)	
			12 (non-smoker firefighter exposed), 9 (non-smoker firefighter control)	–	Non-smoker	Age not well matched between groups (41.0 ± 3.3 vs 47.0 ± 2.6) Small sample size	
			27 (firefighter exposed), 11 (non-smoker general control)	+ ($P < 0.05$)		Exposed firefighter group composed of 15 smokers and 12 non-smokers, while the general controls only non-smokers	
			12 (non-smoker firefighter exposed), 11 (non-smoker general control)	(+)	Non-smoker	Result for this comparison appears significant, but this was not explicitly stated by the authors	

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
DNA strand breaks (alkaline elution)	PBMC	Chemical factory accident (municipal firefighters) Germany, 1993, cross-sectional, 16 male firefighters who worked in a contaminated area after a chemical factory accident for ~8 h without PPE. Samples obtained 19 days (+19 days) and 88 days (+88 days) after exposure; 19 male firefighter trainees (< 2 fires/month) who did not work in the contaminated area, matched on age, alcohol consumption, town of residence, and smoking intensity among smokers; 28 male unexposed non-firefighters, matched on age and smoking intensity among smokers.	16 (paired samples, +16 and +88)	+ ($P < 0.01$) Paired comparison for non-smokers only appears to be significant as well but was not explicitly reported		Unique exposure with limited relevance to the hazards of typical firefighters Exposure assessment: documents likely substantial exposure to quantified mixture of chemicals but no individual exposure measure; contamination exposure status possibly adequate for effect comparisons that were made across groups	Hengstler et al. (1995)
			16 (+19 days exposed firefighters), 19 (trainee firefighters)	+ ($P < 0.05$)	Examined effects of age and alcohol consumption but no significant correlations were observed		
			10 (non-smoking +19 days exposed firefighters), 14 (non-smoking trainee firefighters)	+ ($P < 0.05$)	Non-smoker		
			6 (smoking +19 days exposed firefighters), 5 (smoking trainee firefighters)	–	Smoker	Small sample size	

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
DNA strand breaks (alkaline elution) (cont.)			16 (+88 days exposed firefighters), 19 (trainee firefighters)	–	Examined effects of smoking, age, and alcohol consumption, but no significant correlations were observed		Hengstler et al. (1995) (cont.)
			16 (+19 days exposed firefighters), 28 (non-firefighters)	+ ($P < 0.05$)	Examined effects of age and alcohol consumption, but no significant correlations were observed	Alcohol intake and proportion of smokers to non-smokers in non-firefighters was higher than in exposed firefighter group	
			10 (non-smoking +19 days exposed firefighters), 16 (non-smoking non-firefighters)	+ ($P < 0.05$)	Non-smoker		
			6 (smoking +19 days exposed firefighters), 12 (smoking non-firefighters)	–	Smoker	Small sample size	

Table 4.1 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, date, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
DNA strand breaks (alkaline elution) (cont.)			16 (+88 days exposed firefighters), 28 (non-firefighters)	–	Examined effects of smoking, age, and alcohol consumption, but no significant correlations were observed	Alcohol intake and proportion of smokers to non-smokers in non-firefighters were higher than in exposed firefighter group	Hengstler et al. (1995) (cont.)
		Trainee municipal firefighters	19 (trainee firefighters), 28 (non-firefighters)	–		No specific exposure event; firefighters were trainees and had only participated in < 2 fires/month Alcohol intake and proportion of smokers to non-smokers in non-firefighters was higher than in trainee firefighter group	
DNA adducts (PAH–DNA adducts, ^{32}P-postlabelling)	PBMC	Kuwait oil well fire (volunteers) Kuwait, 1991, cross-sectional; 9 male American volunteers in Kuwait for 6 wk to fight oil well fires. PPE was not used, apart from particle face masks used for up to 2 h/day. Samples collected from volunteers before leaving for Kuwait (pre), and within 3 wk of returning (post) to the USA.	9 (paired samples, pre and post)	–		Small sample size Unique exposure with limited relevance to the hazards of typical firefighters Post-exposure samples obtained up to 3 wk after returning to the USA No exposure assessment. Qualitative exposure assignment based on participant recall; did not account for potentially confounding exposure before the collection of baseline samples	Darcey et al. (1992)

aSPR, age-at-pregnancy standardized prevalence ratio; 1-OHP, 1-hydroxypyrene; ChE, cholinesterase; CI, confidence interval; COSMIC, Catalogue Of Somatic Mutations In Cancer; CYP, cytochrome P450; DSB, DNA strand break; DNMT, DNA methyl transferase; ELISA, enzyme-linked immunosorbent assay; GSTM, glutathione *S*-transferase mu; MDA, malondialdehyde; PAH, polycyclic aromatic hydrocarbon; PBL, peripheral blood lymphocytes; PBMC, peripheral blood mononuclear cells; PM, particulate matter; $PM_{2.5}$, particulate matter with a diameter of ≤ 2.5 μm; PPE; personal protective equipment; PTEN, phosphatase and tensin homologue; PWBC, peripheral white blood cell; S9, 9000 × *g* supernatant; SCE, sister-chromatid exchange; TERT, telomerase reverse transcriptase; U2AF1, serine/arginine-rich splicing factor 2; vs, versus; wk, week; WTC, World Trade Center; yr, year.

[a] +, positive; –, negative; +/–, equivocal; (+), positive result in a study of limited quality.

[b] Factors considered for study quality include the methodology and design, reporting, and quality of exposure assessment.

were pooled and the results were averaged and compared with the post-exposure samples. DNA damage levels were significantly higher in samples obtained after exposure to wood-fuel fires compared with mixed-fuel fires. The level of DNA damage was found to be positively correlated with urinary 1-hydroxypyrene (1-OHP) concentration, skin pyrene concentration, and skin total PAH concentration (see Section 1.4) (Andersen et al., 2018a). [The Working Group noted that the pre-exposure samples were collected 2 weeks before the exposure. Given that all study participants were exposed to fires, and especially given the potential for reduced exposure signal, the Working Group considered the positive result for the 14-day versus post-exposure sample, as well as the significant positive association between DNA damage and PAHs and PAH metabolites, many of which are classified in IARC Group 1, 2A, and 2B, to be particularly informative.]

[The Working Group considered both structure fire studies to be especially informative because of the study design (i.e. pre/post samples and all participants attended fire events), and because both studies detected a significant increase in genotoxicity (urinary mutagenicity and DNA damage in peripheral blood). Moreover, one study demonstrated an association between DNA damage and biomarkers of exposure.]

(ii) Wildland fires

Urinary mutagenicity was evaluated in samples from a population of 19 healthy wildland firefighters (17 men, 2 women) taking part in prescribed burn practices with no respiratory protection in the midwestern region, Ohio, USA (Wu et al., 2020a). Spot urine samples were collected from each study participant immediately before (pre-shift), immediately after (post-shift), and the morning following (the next morning) their shifts. Sampling took place for both prescribed burn (burn day) and regular (non-burn day) work shifts. Burn day study participants had a mean shift length of 4.98 ± 1.34 hours. [The Working Group noted that the shift length for non-burn day study participants was not reported, nor was the interval between the previous prescribed burn shift and the studied burn day or non-burn day shift.] Three different firefighting tasks were recorded: burn day holding (i.e. holding prescribed burn fire lines); burn day lighting (i.e. lighting prescribed burns); and non-burn day (i.e. working at the forest fire office, with few exceptions). Urinary mutagenicity was determined in deconjugated urine concentrates via the plate incorporation version of the Ames/*Salmonella* reverse mutation assay (YG1041 + S9 microsomes). For the samples obtained from firefighters who participated in prescribed burns, the crude (i.e. non-creatinine-adjusted) urinary mutagenic potency in post-shift samples was 156% higher than in the pre-shift samples, but after creatinine adjustment, the change was non-significant (16%, $P = 0.09$). [The Working Group noted that although creatinine adjustment corrects for hydration status, this can be less informative for non-homogeneous study populations since the rate of creatinine excretion has been shown to be affected by gender, and the current study included both men and women. However, the Working Group considered both crude and creatinine-adjusted urinary mutagenicity results to be informative.] For the same burn-day shift participants, there was no significant difference in urinary mutagenic potency between the next-morning samples and the pre-shift samples, without or with creatinine adjustment. For the firefighters who worked a regular (i.e. non-burn day) shift, no significant difference was found in the crude or creatinine-adjusted urinary mutagenic potencies for the post-shift versus pre-shift, or the next-morning versus pre-shift comparisons. [The Working Group noted that, since the non-burn day individuals did not attend prescribed burns, the negative results for non-burn day individuals were not unexpected and demonstrated that the increase in urinary mutagenicity occurred in a

narrow time frame after fire exposure. If samples were collected after the chemicals in the exposure had been excreted, then the mutagenic signal would have been missed.] Across all samples, the cross-shift change in creatinine-adjusted urinary mutagenic potency was significantly associated with the duration of smoke exposure. A linear mixed-effects model was used to examine cross-shift changes in urinary mutagenicity between burn and non-burn days; the authors found pre-shift to post-shift changes in crude values of urinary mutagenicity: levels on burn days were 2.79-fold those on non-burn days. This comparison was no longer significant after creatinine adjustment. The effect of the fire suppression task (i.e. "holding" or "lighting") on cross-shift changes in urinary mutagenicity was also examined. Samples from wildland firefighters who were tasked with "holding" had a pre-shift to next-morning difference in creatinine-adjusted urinary mutagenicity that was 1.56-fold that in firefighters who were tasked with "lighting" during prescribed burns. For the pre-shift to post-shift samples, this comparison was not significant (Wu et al., 2020a). [The Working Group noted that a significant negative correlation was reported between pre-shift to next-morning creatinine-adjusted urinary mutagenic potency and the concentration of black carbon (as measured using a personal sampler) in wildland fire smoke emissions during the prescribed burn. This result suggested that personal exposure measurements of black carbon may not be a good surrogate measure of smoke exposure among exposed firefighters. The Working Group noted that there were no non-firefighter controls in this study.]

In a pilot study from the same group, urinary mutagenicity measured by the plate incorporation version of the Ames/*Salmonella* reverse mutation assay (YG1041 + S9 microsomes) was investigated in samples from 9 male and 3 female healthy non-smoking wildland firefighters from a south-eastern region, South Carolina, USA, taking part in prescribed burn practices with no respiratory protection (Adetona et al., 2019). Spot urine samples were collected from each study participant immediately before (pre-shift), immediately after (post-shift), and the morning following (next morning) their shift. Sampling took place for both prescribed burn (burn day) and regular (non-burn day) work shifts. The mean work shift duration for burn days was 4.5 hours (range, 1.9–9.4 hours), and for non-burn days was 6.2 hours (range, 3.9–7.8 hours). The number of days between the last prescribed burn day shift and the studied work shift was 1 to > 30 days for burn day study shifts, and 3–30 days for non-burn day study shifts. Four different firefighting tasks were recorded: burn day holding (i.e. holding prescribed burn fire lines); burn day lighting (i.e. lighting prescribed burns); non-burn day exposure (i.e. involving occupational exposures to vehicle exhaust, diesel, dust, or smoke from nearby smouldering fires); and non-burn day office (i.e. no reported occupational exposures). No significant differences in the crude or creatinine-adjusted mutagenic potencies were found between post-shift and pre-shift samples, or between next-morning and pre-shift samples for either burn day or non-burn day work shifts. However, the mean cross-shift changes in urinary mutagenicity were routinely higher for burn day samples than for non-burn day samples. There was not a significant difference in the cross-shift crude or creatinine-adjusted urinary mutagenic potency between the different firefighting tasks recorded; however, the "lighting" task consistently had the highest mean cross-shift change in urinary mutagenicity. Significant positive associations were observed between the cross-shift (pre-shift to post-shift) changes in creatinine-adjusted urinary mutagenicity and the concentration of urinary malondialdehyde (a marker of oxidative stress; $P = 0.0010$; see Section 4.1.2), as well as with urinary 1-OHP (a PAH metabolite; $P = 0.0001$); see Section 1.4) (Adetona et al., 2019). [The Working Group noted that consistent

trends in cross-shift urinary mutagenicity were observed and that biomarkers of exposure were associated with urinary mutagenicity. These were both suggestive of an effect of the exposure on urinary mutagenicity; however, this pilot study might be underpowered to obtain statistical significance because of the low sample size. Additionally, the Working Group noted the short interval (i.e. as low as 1 day) between previous burn shifts and the studied burn shifts, the occupational exposures to combustion emissions (including smouldering fire) on the non-burn day shifts, and that no non-firefighter controls were included in this study.]

In a study assessing DNA damage levels using the alkaline comet assay, peripheral blood was obtained from 60 volunteer wildland firefighters in Portugal with ≥ 1 year of experience and 63 office-worker unexposed controls matched by age, gender, and smoking habits (Abreu et al., 2017). Personal protective equipment (PPE) used by firefighters was unknown; this variable was excluded because of the poor response rate for this question on the study questionnaire. The DNA damage level in the firefighters was 76% higher than that in the unexposed controls. These data were then analysed to assess the impact of confounding factors on the level of DNA damage between groups; no significant effect of gender or smoking habits was observed. In addition, a significant positive correlation was found between DNA damage detected using the alkaline comet assay and oxidative lesions detected using the formamidopyrimidine DNA glycosylase (Fpg) version of the comet assay (i.e. Fpg-comet), demonstrating the relationship between these two end-points (see also Section 4.1.2). The study population was subdivided into three age groups to study the influence of age: < 29 years, 29–38 years, and > 38 years. A significant increase in DNA damage in the exposed group compared with the control group was only detected in the age group 29–38 years. In the exposed firefighters, those aged 29–38 years had a significantly higher level of blood DNA damage than did the exposed firefighters aged < 29 years. There was no significant difference between the age group > 38 years and the other two age groups, and no effect of age was found among the control firefighters. The effect of duration of recent firefighting activity on the frequency of DNA damage was investigated, but no significant association was observed. Finally, firefighters were stratified into three groups on the basis of years of service (i.e. < 7 years, 7–15 years, and > 15 years); no statistically significant outcomes were found (Abreu et al., 2017). [The Working Group noted that sampling did not follow a specific exposure event and that the study groups included current smokers, which may reduce the signal-to-noise ratio for genotoxicity induced as a result of wildland firefighting.]

A study in 37 male and 10 female non-smoking wildland firefighters from the USA examined PAH–DNA adduct levels in peripheral white blood cells (Rothman et al., 1993). Samples were taken 8 weeks apart, during the early and late forest fire season. For early and late time-points, respectively, there were 16.0 ± 3.2 hours and 97.4 ± 15.3 hours of self-reported firefighting activity in the 4 weeks preceding blood collection. There was no significant difference in levels of detectable PAH–DNA adducts across the season, and no association was found between the cumulative number of hours of firefighting and levels of PAH–DNA adducts (Rothman et al., 1993). [The Working Group noted that there was no control for consumption of charbroiled food in the early versus late season comparison; however, there was control for this when analysing the association between cumulative hours of recent firefighting activity and DNA adduct levels, and the results were unaffected. The Working Group noted that there were no non-firefighter controls.] In a follow-up study, the same group investigated the impact of *GSTM1* null and *CYP1A1* exon 7 genetic polymorphisms, as well as the interaction between the two polymorphisms and PAH–DNA

adduct levels; no significant results were found (Rothman et al., 1995). There was no association between PAH–DNA adduct levels and cumulative hours of recent firefighting activity either in individual who were *GSTM1* null or in those without this genotype (Rothman et al., 1995). [The Working Group noted that late versus early time-points were not compared within the genotype analysis.] [The Working Group noted that the two studies (Rothman et al., 1993, 1995) on DNA adduct induction after exposure to wildland fire were also informative for the key characteristic of carcinogens "is electrophilic or can be metabolically activated to an electrophile".]

DNA damage was assessed by the alkaline comet assay in peripheral blood collected from 48 exposed non-smoking firefighters, 30 exposed smoking firefighters, and 93 control non-smoking firefighters who did not participate in fire suppression activities, in Portugal (Oliveira et al., 2020). Exposed firefighters participated in wildland fire suppression activities within the 48 hours before sampling, for a median duration of 3 hours. All three groups reported long-term (i.e. median, > 10 years) exposure to forest fire emissions. Only firefighters who did not recently consume grilled, barbecued, or smoked foods were included. There were no significant differences between the three groups in the level of peripheral blood DNA damage detected by the alkaline comet assay. [The Working Group noted that there were no non-firefighter controls or pre-/post-exposure sampling of the same individuals, all three groups reported long-term (i.e. median, > 10 years) exposure to forest fire emissions, and the 48-hour collection window may have been too long to be able to detect DNA damage.]

[The Working Group noted that of the five studies on wildland fires, two gave positive results for genotoxicity. Both positive studies also demonstrated correlations with genotoxicity; one demonstrated a correlation between urinary mutagenicity and biomarkers of exposure, as well as firefighting task, and the other demonstrated a correlation between DNA damage detected in the alkaline comet assay and Fpg-sensitive sites (i.e. oxidative DNA damage) in the blood. Moreover, one of the studies that gave negative results was able to demonstrate a correlation between urinary mutagenicity and biomarkers of exposure, as well as firefighting task. All three of the studies with negative results had methodological issues.]

(iii) Employment as a firefighter

The frequency of micronuclei (MN) in exfoliated buccal epithelial cells obtained from 47 male municipal firefighters in India with ≥ 10 years of service; results were compared with those determined in samples obtained from 40 male office worker controls. The firefighter and control populations were comparable in age distribution, ethnicity, food habits, smoking status and frequency, alcohol consumption, nutritional status, and the extent of indoor air pollution in their homes (Ray et al., 2005). Sample collection from firefighters did not follow a specific exposure event. The frequency of MN in exfoliated buccal epithelial cells from firefighters was 2.1-fold higher than that in matched controls. A significant difference in MN frequency was also found when the firefighters were stratified into two groups by duration of service. The firefighters who had served ≥ 20 years had a mean MN frequency that was 1.4-fold higher than that in firefighters who had served < 20 years (Ray et al., 2005). [The Working Group found this study to be particularly informative because the MN frequency is a more persistent biomarker of genotoxicity than general DNA damage, as well as because of the increased MN frequency observed in firefighters with longer service.]

Peripheral blood lymphocytes obtained from municipal firefighters and matched controls were assessed for the presence of PAH–DNA adducts by quantifying levels of antigenicity for benzo[*a*]pyrene diol epoxide (Liou et al., 1989). There was not a significant increase in the

frequency of PAH–DNA adducts in the DNA of peripheral blood lymphocytes from firefighters compared with the controls before adjustment for confounders, or when adjusted for charbroiled food consumption, alcohol consumption, smoking, or race. When controlling for both charbroiled food intake and race, White firefighters had higher levels of PAH–DNA adducts than did White controls (odds ratio, OR, 3.36; 95% confidence interval, CI, 1.08–10.5; 37 firefighters, 29 controls), but this effect was not significant in non-Whites (6 firefighters, 9 controls). [The Working Group noted the low sample size for non-White study participants.] When controlling for both charbroiled food intake and race, and including an interaction term for firefighting and race, the odds ratio was slightly increased for White study participants (OR, 3.56; 95% CI, 1.04–12.12) (Liou et al., 1989). [The Working Group noted that sample collection did not follow a specific exposure event, the study included current smokers, and that seven control participants had a history of occupational exposure to mutagens. Moreover, the study investigating DNA adducts in exposed humans employed as firefighters was also informative for the key characteristic of carcinogens "is electrophilic or can be metabolically activated to an electrophile".] The study also examined the frequency of sister-chromatid exchange (SCE) in peripheral blood lymphocytes of firefighters and control participants (Liou et al., 1989). Firefighting was not associated with an increase in baseline SCE frequency versus that in controls, including when modelling incorporated the frequency of exposure (i.e. number of fires fought in the last 24 hours, month, or year), or other exposure indices, including use of PPE or duration of employment. No association was found between the frequency of baseline SCE and the frequency of PAH–DNA adducts. [The Working Group noted that sample collection did not follow a specific exposure event, the study included current smokers, and that seven control participants had a history of occupational exposure to mutagens.]

The frequency of SCE in peripheral blood lymphocytes from 9 male non-smoking municipal firefighters and 11 male non-smoking general population controls was investigated (Li et al., 2004). The male non-smoking municipal firefighters had a significantly higher baseline frequency of SCE compared with that in the male non-smoking general population controls (Li et al., 2004). [The Working Group noted that the effect may be confounded by age since the ages of these groups were not well matched (i.e. 47.0 ± 2.6 years for firefighters versus 41.5 ± 2.8 years for general population controls). [The Working Group noted that the male municipal firefighters described above served as an unexposed control group as part of a study investigating male municipal firefighters who were exposed to sarin while responding to the 1997 terrorist attack in Tokyo. The firefighter group reported above was not exposed to sarin.]

Using data gathered as part of the Health and Wellness of Women Firefighters Study, the rate of miscarriage occurring while working in the fire service was evaluated among female firefighters compared with that in age-matched female nurses in the USA (Jung et al., 2021a). Firefighters were identified as study participants if they were working in the fire service when they found out about their pregnancy. Among 1041 pregnant firefighters, 138 experienced a miscarriage (22%). Overall, the age-standardized prevalence ratio for miscarriage was 2.33 (95% CI, 1.96–2.75) in firefighters compared with women from the cohort of nurses in the USA. [The Working Group noted that this constitutes an indirect assessment of genotoxicity. The Working Group also noted study design issues, since firefighters were included in this study if they were active firefighters when they found out they were pregnant, so there was no information regarding the duration of time of active firefighting before or subsequent to finding out they were pregnant.]

In a Danish study, PBMCs were collected from 22 male municipal firefighters before and after a 24-hour work shift (Andersen et al., 2018b). Study participants had 3 days off (rest days) between their last work shift and the studied shift. There was no significant difference in levels of DNA damage, identified by the alkaline comet assay, either across the work shift or when the samples were stratified by participation in fire suppression activities during the work shift (Andersen et al., 2018b). [The Working Group noted that only 14 of the 22 firefighters reported participation in firefighting activities and/or exposure to smoke during the studied work shift, and when samples were stratified by participation in fire suppression activities, the Working Group noted the small sample size (n = 14). The Working Group also noted that the study included current smokers. Both the inclusion of current smokers and the fact that not all firefighters participated in fire suppression activities may reduce the ability to detect a DNA damage signal, given the low prevalence of exposure and that levels of DNA damage are higher in smokers than in non-smokers.]

[The Working Group noted that there were six studies in individuals employed as a firefighter. Four reported genotoxic effects, specifically somatic mutations in cancer-related genes, increased frequency of PAH–DNA adducts and SCE in the blood, together with MN frequency in buccal cells. One study provided indirect evidence of genotoxicity (i.e. miscarriage). The only study in this category that gave negative results used a more transient measure of genotoxicity (i.e. alkaline comet assay in blood), and not all study participants were exposed to fires during the study period.]

(iv) Catastrophic events

The following section describes studies in firefighters who responded to specific emergency response situations resulting from catastrophic events, including the World Trade Center (WTC) disaster on 11 September 2001, "9/11", in New York, USA. [The Working Group noted that these are not typical of firefighting responses and that exposure resulting from these events may not be generally applicable.]

As part of a study in WTC-exposed firefighters compared with non-WTC-exposed firefighters, Jasra et al. (2022) used a deep targeted sequencing approach to analyse 237 genes that are frequently mutated in haematological malignancies. In the firefighter control population (n = 255), the observed mutations were predominantly in *DNMT3A* and *TET2*, both of which are involved in regulating DNA methylation, a process that when dysregulated is known to be associated with cancer (see also Section 4.1.3). Additionally, among the most commonly mutated genes were several known to be associated with cancer (Jasra et al., 2022). [The Working Group noted that there were no non-firefighter controls for this study.]

Jasra et al. (2022) examined the rate of clonal haematopoiesis in whole blood obtained from 429 WTC-exposed firefighters compared with 255 non-WTC-exposed firefighters. Clonal haematopoiesis results from somatic mutations in blood stem cells and is associated with an increased risk of haematological cancer. Using a targeted sequencing approach, the authors analysed 237 genes that are frequently mutated in haematological malignancies. A significantly increased odds ratio of clonal haematopoiesis was found in the WTC-exposed firefighters compared with the non-WTC-exposed firefighters (OR, 2.93; 95% CI, 1.52–5.65) after controlling for age, sex, and race/ethnicity. This result was still significant when the analysis was restricted to study participants with smoking information and controlling for smoking as well as age, sex, and race/ethnicity (OR, 2.78; 95% CI, 1.39–5.59). In the WTC-exposed first responders (i.e. a pooled population of 429 firefighters and 52 emergency medical service workers), mutations were predominantly in *DNMT3A* and *TET2*,

both of which are involved in regulating DNA methylation, a process that when dysregulated is known to be associated with cancer (see also Section 4.1.3). Additionally, mutations (mainly missense) were found in several cancer-associated genes (i.e. *TP53*, *PPM1D*, *STAT3*, *KMT2D*, *U2AF1*, *PTEN*, and *TERT*). [The Working Group noted that mutations were found in many similar genes in the firefighter control group (see above).] Mutation spectrum analysis of samples from the WTC-exposed firefighters revealed enrichment for COSMIC (Catalogue Of Somatic Mutations In Cancer) mutational signatures associated with ageing, DNA mismatch repair, smoking (tobacco), and alkylating agents (COSMIC, 2022). The Working Group also noted that COSMIC mutational signatures were not reported for the non-WTC-exposed firefighters, and that there were no non-firefighter controls.]

A study investigated the frequency of SCE in lymphocytes from 27 male municipal firefighters who were exposed to sarin while responding to the 1997 terrorist attack in Tokyo, Japan, 18 male municipal firefighters (matched on age and smoking status) who were not exposed to sarin, and 11 male non-smoking general population controls (matched on age) (Li et al., 2004). Sarin exposure was confirmed by serum cholinesterase (ChE) activity measured at the time of exposure, then peripheral blood samples were taken 3 years after the Tokyo attack. The exposed firefighters had a significantly elevated frequency of SCE in comparison with both the firefighter control group and the general control group. When controlling for smoking status, the frequency of SCE was significantly higher in exposed firefighter smokers than in control firefighter smokers, but a significant difference was not observed between exposed firefighter non-smokers and the non-smoking control firefighters. [The Working Group noted that there appeared to be a statistically significantly elevated frequency of SCE in the exposed firefighter non-smokers in comparison with the general control non-smokers, but the result of this comparison was not reported by the study authors.] Finally, in the sarin-exposed firefighter group, a significant positive correlation was observed between the frequency of SCE in peripheral blood lymphocytes and the rate of serum cholinesterase (ChE) activity decrease (Li et al., 2004).

An accident in a chemical factory in Germany resulted in the release of a mixture of substances, including *ortho*-nitroanisole, *ortho*-anisidine, and *ortho*-chloronitrobenzene. Peripheral blood samples were collected from one exposed group and two reference control groups, and the alkaline elution assay was carried out on all samples to assess the level of DNA damage (Hengstler et al., 1995). The exposed group was composed of 16 male firefighters who had worked in the contaminated area for approximately 8 hours without PPE, and samples were obtained 19 days and 88 days after the exposure. The first reference group was composed of 19 male firefighter trainees who had not worked in the contaminated area, and as trainees, their previous firefighting activity was low (< 2 fires per month). The second reference group was composed of 28 male non-firefighters with no known occupational exposures to genotoxic substances (Hengstler et al., 1995). A paired analysis of the samples from the exposed firefighters revealed that the mean normalized elution rate for the 19-day samples was significantly higher than for the 88-day samples. The mean normalized elution rate for the exposed firefighters (19-day samples) was statistically higher than that for the unexposed firefighters and the non-firefighter controls. The effect of smoking status on these comparisons was also analysed: the non-smoking exposed firefighters (19-day samples) had significantly more DNA damage than the non-smoking controls in either group, whereas no statistical differences were observed for the smokers. [The Working Group noted the small sample size for the smokers-only analysis.] The normalized elution rate was not significantly different between the two reference

groups. The DNA strand breaks in the 88-day samples were not significantly higher than in either reference group. All firefighters in the exposed group were exposed for approximately 8 hours with a single exception: one individual was exposed for 40 hours. The firefighter exposed for 40 hours had the highest normalized elution rate in the exposed group, and the second highest in the study ($n = 63$) (Hengstler et al., 1995). [The Working Group noted that for the detection of alkali-labile sites, which are representative of transient DNA damage, optimal sample collection would occur within hours rather than days.]

The frequency of PAH–DNA adducts was quantified in PBMC DNA from nine male volunteers who travelled to Kuwait for 6 weeks to fight oil-well fires (Darcey et al., 1992). PPE was not used, apart from particle-filtering face masks used for up to 2 hours per day. Blood samples were collected from volunteers before departure for Kuwait, and within 3 weeks of returning to the USA. Average relative adduct labelling (RAL) was similar for pre- and post-exposure samples; however, for a single study participant, RAL in the post-exposure sample was one-fold higher than that in the pre-exposure sample (Darcey et al., 1992). [The Working Group noted the small sample size, the lack of exposure information, and the fact that post-exposure samples were obtained up to 3 weeks after volunteers returned to the USA, which was probably too long to detect an increase in DNA adducts related to participation in fire suppression in Kuwait.] [The Working Group also noted that the study investigating DNA adducts in exposed humans employed as firefighters was also informative for the key characteristic of carcinogens "is electrophilic or can be metabolically activated to an electrophile".]

(b) Human cells in vitro

See Table 4.2.

(i) Primary human cells

The frequency of SCE induced by wildfire and typical air sample extracts was investigated in lymphocytes obtained from a healthy, non-smoking, male donor aged 25 years. High-volume air samplers were used to collect airborne particles from distant wildfires blown over to the sampling location at the University of Kentucky, USA, and typical air samples were used as control (Viau et al., 1982). A significant concentration-related increase was observed in the frequency of SCE induced by both the "smoky" and "typical" samples. When concentration was expressed per cubic metre of air sampled, the potency of the "smoky" sample was 42-fold higher than that of the "typical" sample. When the concentration units were converted from cubic metres of air sampled to milligram of particles, the "smoky" sample induced approximately 20-fold more SCE than did the "typical" sample and approximately 15-fold more revertants, indicating that the higher potency of the "smoky" sample was related to both the quantity and nature of the PM (Viau et al., 1982).

On 13 September 2001, after the WTC disaster, PM was collected from five locations within 0.5 miles [0.8 km] of ground zero. Human primary lymphocytes were exposed to WTC-PM for 20 hours, and phosphorylated H2A histone family member X (γH2AX) foci accumulation, a biomarker of DNA damage, was assessed by fluorescence microscopy. The samples exposed to WTC-PM showed a statistically significant increase in the percentage of cells containing γH2AX foci in comparison with the untreated control lymphocytes (Jasra et al., 2022). Additionally, the authors examined incorporation of the thymidine analogue 5-ethynyl-2′-deoxyuridine (EdU) by click chemistry to study the effect of treatment with WTC-PM on cell cycle progression through S-phase. Lymphocytes treated with WTC-PM did not display a significant increase in the number of EdU-positive

Table 4.2 Genetic and related effects in human cells in vitro

End-point	Tissue, cell line	Test material	Results[a]		Concentration (LEC or HIC)	Comments[b]	Reference
			Without metabolic activation	With metabolic activation			
Primary human cells							
SCE	Human primary lymphocytes	Organic extracts of airborne particles from distant wildfires ("smoky" sample)	+	NT	Air, 16.4 m^3/flask [PM, 3 mg/flask]	Sample potency was 43-fold that of a control typical air sample (in SCE/cell per m^3); when the unit was converted (SCE/cell per mg PM), it was 21-fold	Viau et al. (1982)
γH2AX	Human primary lymphocytes	WTC-PM collected from 5 locations within 0.5 miles [0.8 km] of ground zero on 13 September 2001	+ ($P < 0.0001$)	NT	PM, ≤ 200 µg/mL	Only a single concentration tested Size of PM not described	Jasra et al. (2022)
Cell cycle dysregulation (EdU-incorporation)			– Accumulation of cells in mid to late S-phase was observed but no statistical test result was reported	NT			
Common fragile sites			+ ($P < 0.05$) Significantly altered replication programme, including replication pausing, increase in initiation events, and a significant increase in replication speed	NT			

Table 4.2 (continued)

End-point	Tissue, cell line	Test material	Results[a]		Concentration (LEC or HIC)	Comments[b]	Reference
			Without metabolic activation	With metabolic activation			
Human cell lines							
DNA damage (alkaline comet assay)	Human lung epithelial carcinoma, A549	PM collected in a fire house during a firefighter rescue educational course; samples collected for 7 h/day over 2 days during smoke diving exercises with combustion of standard wooden pallets in the absence or presence of foam mattresses and electrical cords	–	NT	PM, 100 µg/mL	Unwinding/electrophoresis buffer pH not reported, authors used a manual arbitrary scoring scale No metabolic activation	Ma et al. (2020)
Micronucleus frequency	Human lung epithelial carcinoma, A549	EOM from PM_{10} + aerosols collected from biomass burning during the dry season (i.e. intense burning) of 2011 in the Amazon	+	NT	EOM, 100 µg/mL		de Oliveira Galvão et al. (2018)
			+	NT	EOM, 50 µg /mL		

EdU, 5-ethynyl-2′-deoxyuridine; EOM, extractable organic material; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; PM, particulate matter; SCE, sister-chromatid exchange; WTC, World Trade Center.

[a]+, positive; –, negative.

[b] Factors considered for study quality include the methodology and design, and reporting.

cells. S-phase cells were further characterized as to what stage they were in (i.e. early, mid, or late S-phase). The authors reported that treatment with WTC-PM resulted in an accumulation of cells in mid to late S-phase, indicating that treatment increased the rate at which cells progress through S-phase (Jasra et al., 2022). [The Working Group noted that statistical results were not reported for this analysis, although a shift in cell populations did seem apparent.] Finally, Jasra et al. (2022) examined WTC-PM-induced effects at common fragile sites, which are genomic hotspots of replication stress. WTC-PM-treated lymphocytes showed a significantly altered replication programme, which included multiple sites of replication pausing, a significant increase in initiation events, and a significant increase in the speed of the replication fork. [The Working Group noted that PM size was not described, and it was unclear how sterility was maintained with PM exposures for 20 hours.]

(ii) Human cell lines

PM was collected using an electrostatic sampler placed in a fire house during the firefighter rescue educational course described above in the study by Andersen et al. (2018a). Samples were collected for 7 hours per day over 2 days during smoke diving exercises involving combustion of standard wooden pallets in the absence or presence of foam mattresses and electrical cords. Induced DNA damage was assessed in cultured human adenocarcinoma cells (A549) using the alkaline comet assay after a 3-hour exposure to PM. No significant treatment effects were observed for PM samples produced with either type of combustion fuel (Ma et al., 2020). [The Working Group noted that the authors did not test the PM in the presence of exogenous metabolic activation.]

Pooled extractable organic material (EOM) from PM_{10} (diameter, ≤ 10 μM) and aerosol samples collected during prescribed burns in the Amazon, Brazil, was assessed for clastogenicity using the MN assay in human A549 cells (de Oliveira Galvão et al., 2018). Samples were collected during both the dry season of 2011 (i.e. intense biomass burning) and wet season of 2011–2012 (i.e. moderate biomass burning). A concentration-dependent increase in the frequency of MN was observed for EOM samples from both the dry season (moderate) and wet season (intense) burning. There was no statistical difference between the MN responses induced by samples collected in the dry season and those collected in the wet season. [The Working Group noted that the authors did not use clean air control samples as a reference.]

(c) Experimental systems

(i) Non-human mammals in vivo

Using the WTC-PM previously described in Jasra et al. (2022), C57BL/6 mice were exposed to a single administration of either phosphate buffered saline (PBS) or 100 μg of WTC-PM (collected from five locations within 0.5 miles [0.8 km] of ground zero) in PBS by oropharyngeal aspiration, with humane killing of animals taking place after 30 days. DNA was isolated from bone marrow cells and used for whole-genome sequencing. Exposure to WTC-PM induced a significant increase in the frequencies of non-synonymous SNPs (single nucleotide polymorphisms, $P = 0.03$), deletions ($P = 0.007$), and indels (small insertions and deletions, $P = 0.046$). [The Working Group noted that the result for insertions alone was not reported; however, this did not appear to be significant unless combined with deletions (i.e. for indels).] Murine mutational signatures were determined after further analysis of the detected SNPs and were compared with the COSMIC human mutational signatures. Murine signatures in bone marrow of WTC-PM-exposed mice were closely matched to the COSMIC signatures for tobacco smoking (SBS04) and defective homologous recombination DNA damage repair (SBS03) (COSMIC,

2022). Additionally, bone marrow cells were sorted by flow cytometry to isolate haematopoietic stem cells (i.e. KSL stem cells). An expansion of the haematopoietic stem cell population was observed in the WTC-PM-treated animals, in comparison with the vehicle control group (Jasra et al., 2022). [The Working Group noted that the size of the PM was not described.]

(ii) Bacteria

See Table 4.3.

Organic extracts of combustion emissions relevant to the occupational exposure of firefighters have been evaluated in two studies using *Salmonella typhimurium* tester strains sensitive to frameshift mutations (i.e. TA98) and to base-pair substitutions (i.e. TA100). The organic extracts from the "smoky" and "typical" air samples, as described in the study by Viau et al. (1982) on wildland fires in Kentucky, USA, were assessed for mutagenicity using the plate incorporation version of the Ames/*Salmonella* reverse mutation assay. The extract from the "smoky" sample gave positive results in TA98 with and without metabolic activation (i.e. S9), and in TA100 without S9 metabolic activation. It was marginally positive in TA100 with S9 metabolic activation. In comparison with the "typical" extract, the "smoky" extract was more potent under all tested conditions, whether the dose unit was presented in terms of cubic metres of air or in terms of micrograms of particles, indicating that the observed genotoxicity was related to both the quantity and nature of the particles. For the "smoky" sample, TA98 was the more sensitive strain, indicating predominantly frameshift mutations, and testing with S9 was more sensitive for the detection of mutations than testing without, indicating that the mutagens require metabolic activation (Viau et al., 1982). Another study examined the bacterial mutagenicity of condensates produced from the oxidative pyrolysis of four polyamides and polyvinyl chloride (PVC), from various industrial areas in France (Chastagnier et al., 1991). Polyamides (also known as nylons) are used in textile, plastic, electronic, automotive, and sporting equipment industries, among others, because of their many desirable properties, which include high tensile strength, flexibility, and heat resistance. The authors found that PVC and all four tested polyamide concentrates gave positive results in the pre-incubation version of the Ames/*Salmonella* assay in both TA98 and TA100 with S9. As with the above study, TA98 was more sensitive than TA100 for all tested condensates, indicating that the mutagenic compounds induce primarily frameshift mutations, and testing with S9 was more sensitive than without, indicating that the mutagens require metabolic activation (Chastagnier et al., 1991).

The EOM from the biomass burning samples from the Amazon, Brazil, described above in the study by de Oliveira Galvão et al. (2018) was also assessed for mutagenicity in the Ames/*Salmonella* assay in both TA98 and YG1041, with and without S9. YG1041 is derived from TA98 strain but contains a plasmid carrying genes encoding nitroreductase and acetyltransferase enzymes. Positive responses were observed for all tested conditions. The EOM from the dry season samples (i.e. intense burning) was more potent than the EOM from the wet season samples (i.e. moderate burning) in both strains. The most potent response was observed in YG1041 without S9. In TA98, the response was approximately equally potent with and without S9. Taken together, the mutagenic responses observed in this study were induced by both directly and indirectly acting frameshift mutagens, and the YG1041 response indicated a contribution from directly acting nitroaromatic compounds. [The Working Group noted that de Oliveira Galvão et al. (2018) measured nitro-PAHs in the EOM samples, which corroborates this statement. The Working Group also noted that the authors did not use clean air control samples as a reference.]

Table 4.3 Genetic and related effects in bacterial experimental systems

Test system (species, strain)	End-point	Test agent	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98	Reverse mutation	Organic extracts of airborne particles from distant wildfires ("smoky sample")	+	+	1.02 m^3 air/plate [PM, 188 µg/plate] without activation, 2.03 m^3 air/plate [PM, 376 µg/plate] with activation	The "smoky" extract was more potent than the "typical" air extract, up to 38-fold in rev/plate per m^3 of air (16-fold in rev/µg PM per plate)	Viau et al. (1982)
Salmonella typhimurium TA100		Organic extracts airborne particles from distant wildfires ("smoky sample")	+	(+)	4.07 m^3 air/plate [PM, 753 µg/plate] without and with activation	The "smoky" extract was more potent than the "typical" air extract, up to 18-fold in rev/plate per m^3 of air (6-fold in rev/µg PM per plate) Result with metabolic activation considered marginally positive as dose-related increase was observed but only reached 1.8-fold the control value	

Table 4.3 (continued)

Test system (species, strain)	End-point	Test agent	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98 (pre-incubation)	Reverse mutation	Condensate from oxidative pyrolysis of polyvinyl chloride	+	+	NR	Potency reported but not individual test concentrations	Chastagnier et al. (1991)
		Condensate from oxidative pyrolysis of polyamide 6	+	+	NR	Potency reported but not individual test concentrations	
		Condensate from oxidative pyrolysis of polyamide 6–10	–	+	NR	Potency reported but not individual test concentrations	
		Condensate from oxidative pyrolysis of polyamide 11	–	+	NR	Potency reported but not individual test concentrations	
		Condensate from oxidative pyrolysis of polyamide 6–6	+	+	NR	Potency reported but not individual test concentrations	
Salmonella typhimurium TA100 (pre-incubation)		Condensate from oxidative pyrolysis of polyvinyl chloride	+	+	NR	Potency reported but not individual test concentrations	
		Condensate from oxidative pyrolysis of polyamide 6	–	+	NR	Potency reported but not individual test concentrations	
		Condensate from oxidative pyrolysis of polyamide 6–10	–	+	NR	Potency reported but not individual test concentrations	
		Condensate from oxidative pyrolysis of polyamide 11	–	+	NR	Potency reported but not individual test concentrations	
		Condensate from oxidative pyrolysis of polyamide 6–6	+	+	NR	Potency reported but not individual test concentrations	

Table 4.3 (continued)

Test system (species, strain)	End-point	Test agent	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98	Reverse mutation	EOM from PM_{10} + aerosols collected from biomass burning during the dry season (i.e. intense burning) of 2011 in the Amazon	+	+	25 µg EOM/plate without activation, 12.5 µg EOM/plate with activation		de Oliveira Galvão et al. (2018)
Salmonella typhimurium TA98		EOM from PM_{10} + aerosols collected from biomass burning during the wet season (i.e. moderate burning) of 2011 in the Amazon	+	+	5 µg EOM/plate without activation, 50 µg EOM/plate with activation		
Salmonella typhimurium TA100		EOM from PM_{10} + aerosols collected from biomass burning during the dry season (i.e. intense burning) of 2011 in the Amazon	+	+	50 µg EOM/plate without activation, 250 µg EOM/plate with activation		
Salmonella typhimurium TA100		EOM from PM_{10} + aerosols collected from biomass burning during the wet season (i.e. moderate burning) of 2011 in the Amazon	+	+	250 µg EOM/plate without activation, 500 µg EOM/plate with activation		

EOM, extractable organic material; HIC, highest ineffective concentration; LEC, lowest effective concentration; PM, particulate matter; PM_{10}, particulate matter with diameter ≤ 10 µm; NR, not reported; rev, revertants.

[a] +, positive; (+), positive in a study of limited quality.

4.1.2 Induces oxidative stress

(a) Exposed humans

See Table 4.4.

A group of studies assessed the association between oxidative stress and firefighting in exposed humans, with a wide variety of end-points measured. End-points included those that are indicative of oxidative DNA damage, such as oxidized guanine species (Ox-GS), including 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). Ox-GS are formed during oxidized DNA repair and therefore act as biomarkers for acute redox activity. Oxidative DNA damage was also assessed via the comet assay with measurement of formamidopyrimidine DNA glycosylase (Fpg), providing detail on the concentration of DNA oxidized purines. Other biomarkers measured can be formed into two categories, antioxidants and markers of free radical activity or damage, since oxidative stress is the result of an imbalance between antioxidant capacity and free radicals. Antioxidant-related biomarkers included in these studies are: catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione reductase (GR), superoxide dismutase (SOD), thiol groups, total antioxidant activity, trolox equivalent antioxidant capacity (TEAC), total radical-trapping antioxidant potential (TRAP), and uric acid (UA). Reactive oxygen species (ROS) and damage markers included are: 8-isoprostane, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF2α), advanced oxidation protein products (AOPP), receptor for advanced glycation end-products (RAGE), conjugated diene, disulfide, dichlorofluorescein (DCF), gamma glutamyl transpeptidase (GGT), oxidized glutathione (GSSG), hydrogen peroxide (H_2O_2), lipid hydroperoxides (LOOH), malondialdehyde (MDA), myeloperoxidase (MPO), 3-nitrotyrosine (3-NT), and protein carbonyls (PC).

Biomarkers of oxidative stress were investigated in relation to a variety of exposure types; these included: structure fire exposures, wildland fire exposures, firefighters with a history of unclassified exposures, and acute exercise or smoke exposure with no fire suppression activities.

(i) Structure fires

Two studies (Andersen et al., 2018a; McAllister et al., 2018) assessed structure training fire exposures, and three studies (Al-Malki et al., 2008; Keir et al., 2017; Andersen et al., 2018b) investigated the consequence of operational structure fires on oxidative stress.

PBMCs collected from trainee firefighters from Denmark 14 days before, immediately after, and 14 days after exposure during a 3-day training course revealed a significant increase in oxidative DNA damage (i.e. Fpg-sensitive sites) in samples collected immediately after exposure compared with those collected before but not 14 days after exposure. A non-significant trend was observed for increased Fpg-sensitive sites in samples collected after exposure to fires with wood fuel in comparison with mixed-fuels. The frequency of Fpg-sensitive sites was positively correlated with skin total PAH concentration, but not with urinary 1-OHP (Andersen et al., 2018a). [The Working Group noted that this study was particularly informative because of the large sample (n = 53) of non-smoking participants, pre/post design, and the significant association between oxidative DNA damage and PAH content of skin wipes from the neck. Findings may suggest that fuel type may be a contributory factor to oxidative stress occurrence.] In a study performed in the USA, training fire search and rescue (~17–20 minutes) within a heat house resulted in no association with GSSG, GSH/GSSG, or SOD levels, but caused increased CAT and decreased AOPP levels; the antioxidant supplement curcumin had no effect (McAllister et al., 2018) [The Working Group considered that

Table 4.4 End-points relevant to oxidative stress in exposed firefighters

Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments[b]	Reference
Structure fires						
Blood (PBMC)	Training (3-day course) Denmark, pre/post study trainee male and female firefighters, repeated measures design 14 days before, immediately post-, and 14 days post-exposures	53 (19 exposed to wood combustion, 34 exposed to wood, foam mattresses and electrical cord combustion)	↑ Fpg-sensitive sites post vs pre ($P < 0.05$) ↑ Fpg-sensitive sites immediately post vs combined pre and 14 days post ($P < 0.05$) Fpg-sensitive sites positively correlated with skin total PAH concentrations	Non-smokers, same supply of food	Limited age range of participants (18–26 yr); PPE and breathing apparatus worn; comet scoring carried out by visual classification Exposure assessment: appropriate personal shift PAH and 1-OHP exposure measures; firefighting was appropriately evaluated as exposure in the pre/post design	Andersen et al. (2018a)
Blood	Training (heat house, victim search and clear) USA, male firefighters, pre/post trial, repeated measures design, exposure with fire vs exposure without fire	10	↑ GSH greater at all time-points (including pre) with fire ($P < 0.05$) No change in GSSG, GSH/GSSG, SOD pre- to post-exposure both with and without fire ↑ CAT both with and without fire ($P = 0.005$) ↓ AOPP 30 min post exercise, both with and without fire ($P = 0.0009$)	Smoking habits, cardiovascular diseases	Randomized to job role within task; small sample size Exposure assessment: exposure to heat appropriately tested as exposure for the effects assessments that were done in the experiment	McAllister et al. (2018)

Table 4.4 (continued)

Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments[b]	Reference
Blood (PBMC)	Multi (24-h shift included car, basement, waste container, apartment fires) Denmark, male firefighters, pre/post	22 (14 exposed, 8 non-exposed)	↓ in Fpg-sensitive sites after shift for all participants ↓ in Fpg associated with fire suppression activities	No exposure for 3 days prior; similar timing of cross-shift sample collection	Small sample size; underpowered for statistical analysis; study included smokers; comet scoring carried out by manual visual classification Exposure assessment: firefighting was appropriately evaluated as exposure (PAH, 1-OHP measures) in the pre/post design; other exposure measures apparently not used in effect analysis; some logistic difficulties	Andersen et al. (2018b)
Urine	Residential or commercial operational fire Canada, male firefighters, pre/post and comparison to office worker controls	16 (31 pre and post sample pairs), 17 (18 samples)	No change in 8-iso-PGF2α pre to post	Smoking habit, non-exposure combustion sources, age, urine dilution (creatinine adjustment)	Small sample size given possible variability in operational fire roles and exposure duration; end-point may be altered by oxygen availability via breathing apparatus Exposure assessment: appropriate personal shift PAH exposure measure; firefighting was appropriately evaluated as exposure in the pre/post design	Keir et al. (2017)

Table 4.4 (continued)

Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments[b]	Reference
Blood	Operational fire (no other detail) Saudi Arabia, male firefighters, cross-sectional, firefighters from 2 locations vs non-exposed control	37 (28, 9), 9	No change in GGT	Cardiovascular disease, sample collection timing	GGT showed non-significant increase, but firefighter groups were not combined in the analysis, possibly underpowered; limited sample analysis detail Exposure assessment: temporality issue is somewhat handled by collection of samples among firefighters within first hour after firefighting	Al-Malki et al. (2008)
Wildland fires						
Blood	Training (wildland fire exposure as part of 2 wk pre-season training) USA, pre/post study, male and female wildland firefighters, repeated measures design, day 1 vs day 4 vs day 8 vs day 11	18 men and 3 women	↑ LOOH day 4, 8, 11 vs day 1 ($P < 0.05$) ↑ 3-NT day 8 vs day 4 ($P < 0.05$) ↓ 8-Isoprostane day 4 and 8 vs 1 ($P < 0.05$) No change in PC	Sample timing	Variability of training tasks; limited detail of fire exposure; limited detail regarding participant health (smoking habit, cardiovascular disease); no non-exposed controls Exposure assessment: Specific firefighting exposure was not evaluated but effect of involvement in firefighting appropriately tested with the study design	Gurney et al. (2021)
Exhaled breath condensate	Wildland prescribed burn USA (south-eastern), male and female wildland firefighters, pre/post, immediately post and morning after exposure compared for day type (exposure vs control)	12 (84 exposure sample sets),12 (36 non-exposure sets)	No change in 8-isoprostane pre- to post-exposure or between exposure and control at any time-point	Cardiovascular and respiratory diseases	No detail of control non-burn day activity Exposure assessment: firefighting appropriately used for analysis in the pre/post comparisons; no personal monitoring data was used in analysis	Wu et al. (2020b)

Table 4.4 (continued)

Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments[b]	Reference
Urine	Wildland prescribed burn USA (midwest), male and female wildland firefighters, pre/post, immediately post- and morning after exposure compared for day type (exposure vs control) and work task (holding fire, lightly fire, non-burn exposure, non-burn office work)	19 (81 pre- and post-exposure sample pairs), (39 non-exposure pairs)	↑ Ox-GS next morning compared with pre with exposure ($P = 0.03$) ↑ 8-Isoprostane and Ox-GS changes greater on burn than non-burn days ($P = 0.03$ and 0.02, respectively) No change in MDA Positive correlation between change in MDA and black carbon ($P = 0.01$)	Urinary dilution (creatinine adjustments)	Non-burn exposure day tasks may lead to misclassification of exposure Exposure assessment: firefighting and shift personal exposure to $PM_{2.5}$ and black carbon appropriately used for analysis in the pre/post comparisons	Wu et al. (2020a)
Urine	Wildland prescribed burn USA (south-eastern), male and female wildland firefighters and volunteers, pre/post, immediately post- and morning after exposure compared for day type (exposure vs control) and work task (holding fire, lightly fire, non-burn exposure, non-burn office work)	12 (10 firefighter, 2 volunteers; 48 pre- and post-exposure sample pairs, 40 including morning after), 8 (19 pre- and post-non-exposure pairs, 16 including morning after)	No change in 8-isoprostane or MDA Positive correlation between MDA change and 1-OHP ($P = 0.0001$)	Chewed tobacco, age, career length, shift duration, days since last burn, urinary dilution (creatinine adjustments)	Small sample size; non-burn exposure day tasks may lead to misclassification of exposure; sample analysis blinded Exposure assessment: appropriate personal shift $PM_{2.5}$, black carbon, and 1-OHP exposure measures; firefighting was appropriately evaluated as exposure in the pre/post design	Adetona et al. (2019)

Table 4.4 (continued)

Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments[b]	Reference
Urine	Wildland fire (2 days of 12.5 h) USA, male wildland firefighters, cross-sectional, non-exposed vs exposed from recent 5 days	20, 18	↑ 8-OHdG in exposed ($P = 0.01$), although not significant when controlled for levoglucosan ($P = 0.07$) No change in 8-isoprostane	Smoking, urine dilution (creatinine adjustment)	Asthma reported was physician diagnosed; no control for diet levoglucosan; no pre-exposure sample collection; limited detail regarding non-exposed firefighter tasks Exposure assessment: levoglucosan concentrations may not well reflect variability in exposure between firefighters	Gaughan et al. (2014b)
Blood (PBMC)	Wildland (forest) fire Portugal, firefighters, cross-sectional, non-smokers exposed vs smokers exposed vs station control	48 (non-smokers exposed), 30 (smokers exposed), 93	↑ Net-Fpg in non-smokers exposed vs control ($P < 0.001$) and smokers ($P < 0.05$) Positive correlation Net-Fpg with urinary 2-OHF and 1-OHP ($P < 0.05$)	Smoking habits, diet, cardiovascular diseases	Post samples collected at end of shift, exposure time varied from 2 to 12 h, time from end of exposure to sampling was unclear; unclear if male and/or female participants Exposure assessment: firefighting status used for comparison with controls and biomonitoring data used for correlation analysis limited because of only post-exposure collection	Oliveira et al. (2020)

Table 4.4 (continued)

Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments[b]	Reference
Urine	Wildland prescribed burn USA (south-eastern), male and female wildland firefighters, pre/post, model analysis of end-point changes with $PM_{2.5}$ exposure, career length, and age	17 (providing 94 pre and post sample pairs)	↑ 8-OxodG pre to post with ≤ 2 yr career length ($P = 0.04$). ↓ 8-OxodG pre to post with ≥ 10 yr ($P = 0.03$) MDA: no association with age, career length, or $PM_{2.5}$	Second-hand smoke exposure, smoking, urinary dilution (creatinine adjustments)	Large variation in number of samples provided per participant; pre and post samples with different time conditions and undefined period between burn days Exposure assessment: appropriate personal shift $PM_{2.5}$ exposure measure; firefighting was appropriately evaluated as exposure in the pre/post design	Adetona et al. (2013)
Blood (PBMC)	History of wildland exposure Portugal, volunteer firefighter (male and female) and non-exposed office workers, cross-sectional	60, 63	No change in Net-Fpg Positive correlation between comet assay-detected DNA strand breaks and Net-Fpg ($P < 0.05$)	Age, gender, smoking habits, BMI, respiratory pathologies, recent exposures	Not controlled for PPE use; limited statistical analysis data presented; sample analysis blinded Exposure assessment: no information on specific exposures	Abreu et al. (2017)
Employment as a firefighter						
Urine	Operational fire (type not defined) Republic of Korea, male firefighters, cross-sectional, exposed ≥ 8 h in 5 days vs exposed < 8 h in 5 days vs non exposed	49 (13 ≥ 8 h, 36 < 8 h), 24	No change in 8-OHdG	Smoking, diet, age, BMI, urine dilution (creatinine adjustment)	No detail on type of fire Exposure assessment: misclassification of length of time unlikely, but non-consideration of intensity (amount of exposure) could be an issue	Hong et al. (2000)

Table 4.4 (continued)

Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments[b]	Reference
Blood	History of exposure. Shift week (but type not clearly defined) Türkiye, male firefighters vs office worker controls, cross-sectional	100, 50	↑ Disulfide in firefighters ($P < 0.001$) ↑ Disulfide:thiol % ratio ($P < 0.001$)	Cardiovascular disease, antioxidant supplements, smoking habit	Samples collected at end of shift week, no control for time since recent exposure, recent exposure number, physical activity or diet; no measurement post exercise or fire exposure Exposure assessment: Employment as a firefighter possibly adequate for effects comparisons that were made; rationale for choice of arsenic uncertain	Gündüzöz et al. (2018)
Exposure to heat, mental, or physical challenges						
Blood	No fire exposure (strength, anaerobic, and aerobic fitness test) Brazil, male military firefighters, pre/post treadmill fitness test, RCT	30 (with resveratrol), 30 (without resveratrol)	No change in all parameters pre- to post-exposure fitness test	Energy intake before exercise	Unknown firefighting exposure history; no heat/live fire/PPE Exposure assessment: engagement in experimental fitness test appropriately tested as exposure for the effects assessments that were done in the experiment; compliance with taking capsule was not reported	Macedo et al. (2015)
Blood	No fire exposure (treadmill exercise in temperate environment) Republic of Korea, male volunteer firefighters, pre/post treadmill exercise in PPE vs regular clothing, 25 °C at 9 METs	12 (PPE),12 (regular clothing)	↑ Exercise in PPE increased CD ($P < 0.05$) and TRAP ($P < 0.01$) No change in SOD, GSH-Px, or CAT	Cardiovascular disease, antioxidant nutrient intake	No heat/live-fire exposure; small sample size; limited ecological validity to firefighter tasks; no detail of regular clothing; no statistical comparison between PPE and regular clothing trials Exposure assessment: PPE-wearing appropriately tested as exposure for the effects assessments that were done in the experiment	Park et al. (2016)

Table 4.4 (continued)

Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments[b]	Reference
Blood	No fire exposure (6-week training programme) USA, male firefighters, pre/post time-restricted feeding (TRF) over 6 wk	15 (pre vs post)	↓ TRF decreased AOPP ($P = 0.02$) and AGE ($P = 0.05$)	Diet, training status, cardiorespiratory diseases	Sequential design without control group; no measurement post exercise or fire exposure Exposure assessment: firefighting-specific exposure was not assessed	McAllister et al. (2020)
Exhaled breath condensate, blood	Wood smoke (treadmill exercise in temperate environment with wood smoke) USA, male firefighters, pre/post randomized cross-over, filtered air vs wood smoke	10 (pre vs post); no control group	↓ Immediately post-exposure 8-isoprostane was lower than in filtered air ($P < 0.05$) ↑ 1 h post-exposure 8-isoprostane increased compared with filtered air ($P < 0.05$) No change in MPO or H_2O_2	Similar timing of data collection, fitness, smoking habits	No details on clothing worn; shorter duration exposure than wildland fire; small sample size Exposure assessment: the experimental exposure to different concentrations appropriate for the pre/post design	Ferguson et al. (2016)

Table 4.4 (continued)

Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments[b]	Reference
Blood	Wood smoke (treadmill exercise in temperate environment with smoke) USA, experimental RCT, clean air vs wood smoke low (250 μg/m^3) vs woodsmoke high (500 μg/m^3) $PM_{2.5,}$ pre, post, 1 h post	10 (pre vs post); no control group.	↓ UA post combined smoke exposure ($P = 0.032$) ↑ TEAC post vs pre for both clean air ($P = 0.015$) and high exposure ($P = 0.001$) and 3-NT post vs pre for combined smoke exposure ($P = 0.049$) ↓ LOOH 1 h post high smoke exposure ($P = 0.036$) ↑ 8-Isoprostane and MPO with low ($P = 0.004$, $P = 0.035$) and high ($P = 0.009$, $P = 0.019$) exposure No change in PC	Similar timing of data collection, respiratory disease, wood smoke exposure, fitness level	Some statistical comparisons to control unclear; no details on clothing worn; shorter duration exposure than wildland fire; small sample size Exposure assessment: exposure was relative to wildfire situation but exposure vs non-exposure to woodsmoke appropriately tested for the assessment of effects	Peters et al. (2018)

AGE, advanced glycated end-products; AOPP, advanced oxidation protein products; BMI, body mass index; CAT, catalase activity; CD, conjugated diene; DCF, dichlorofluorescein; Fpg, formamidopyrimidine DNA glycosylase; GGT, gamma glutamyl transpeptidase; GR, glutathione reductase; GSH, glutathione; GSH-Px, glutathione peroxidase activity; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; 8-iso-PGF2α, 8-iso-prostaglandin $F_{2α;}$ LOOH, lipid hydroperoxides; MET, maximal exercise treadmill training; MDA, malondialdehyde; MPO, myeloperoxidase; 3-NT, 3- nitrotyrosine; 2-OHF, 2-hydroxyfluorene; 8-OHG, 8-hydroxyguanosine; Ox-GS, oxidized guanine species; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; PAH, polycyclic aromatic hydrocarbon; PBMC, peripheral blood mononuclear cells; PC, protein carbonyls; PM, particulate matter; PPE, personal protective equipment; RCT, randomized controlled trial; SOD, superoxide dismutase; TEAC, trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidant potential; TRF, time restriction feeding; UA, uric acid; vs, versus; yr, year.

[a], ↑, increase; ↓, decrease.

[b] Factors considered for study quality include the methodology, design, reporting, and quality of exposure assessment.

this small sample included firefighter participants who were healthy with consistently high levels of physical activity; consequently they may not provide a valid reflection of the general firefighter population. As noted in Section 1.2, there may be a similar or greater prevalence of obesity in firefighters compared with the general population.]

Assessment of PBMCs collected from operational firefighters from Denmark across shifts indicated a decrease in the frequency of Fpg-sensitive sites, using the Fpg-comet assay, after a 24-hour work shift and when compared with PBMCs from non-exposed firefighters on the same shifts (Andersen et al., 2018b). [The Working Group noted the small sample size (14 out of 22 participants exposed to fire) and the inclusion of current smokers as participants. These factors may have reduced the ability to detect oxidative DNA damage, given the lower prevalence of exposure and the association between smoking and increased oxidative DNA damage.] Emergency structure fire suppression has also been reported to result in no cross-shift changes in urinary 8-iso-PGF2α (Keir et al., 2017). [The Working Group highlighted the fact that 8-iso-PGF2α levels may be altered by the hyperoxic conditions resulting from breathing apparatus use.] A further assessment of operational fires revealed that post-exposure levels of serum GGT were elevated in comparison to non-exposed controls, but not significantly (Al-Malki et al., 2008). [The Working Group considered that the absence of pre-exposure samples and details of fire exposure type limited the conclusions that could be drawn from this result.]

(ii) Wildland fires

Effects on oxidative stress markers were assessed in one study on wildland fire training (Gurney et al., 2021), and a further five studies on acute wildland fire exposures (Adetona et al., 2013, 2019; Gaughan et al., 2014a; Wu et al., 2020a, b). Two additional cross-sectional studies compared wildland firefighters with non-exposed controls (Abreu et al., 2017; Oliveira et al., 2020).

Wildland fire training resulted in decreased levels of 8-isoprostane, no change in PC, and increases in levels of plasma LOOH and 3-NT (Gurney et al., 2021). [The Working Group noted that minimal exposure details were provided for the wildland training.] Cross-shift assessment and comparison of exposure with non-exposure days revealed no significant changes in urinary or exhaled breath condensate (EBC) 8-isoprostane by enzyme-linked immunosorbent assay (ELISA) analysis (Gaughan et al., 2014a; Adetona et al., 2019; Wu et al., 2020b), although Wu et al. (2020b) did report a marginal but non-significant cross-shift increase in levels of 8-isoprostane on burn days. Gaughan et al. (2014a) reported elevated levels of 8-OHdG as measured by ELISA urine analysis in firefighters after recent fire suppression activities compared with firefighters with no recent exposure; however, after adjusting for urinary levoglucosan, which is a cellulose pyrolysis product that may indicate smoke exposure, differences were no longer present. [The Working Group noted that a major contributor for levoglucosan is also diet, which was not controlled for.] Alternately, a positive correlation was noted between pre- and post-wildland exposure changes in urinary MDA levels and exposure markers (1-OHP), despite no significant change in MDA levels (Adetona et al., 2019) (see Section 4.1.1). [The Working Group noted variations in the details provided regarding tasks completed on non-exposure days and sample collection time-points; also, timing of sample collection by Wu et al. (2020b) may not have been optimal for 8-isoprostane measurement.]

A more comprehensive analysis of creatinine-corrected oxidative stress markers in urine after wildland fire exposure revealed increases in Ox-GS the morning following the burn compared with pre-exposure levels; Ox-GS and

8-isoprostane changes were also greater on burn days compared with non-burn days (Wu et al., 2020a). Biomarkers were analysed by ELISA, with Ox-GS analysed as a combined ELISA including 8-OHdG, 8-hydroxyguanosine (8-OHG), and 8-hydroxyguanine (8-OHGua). A positive correlation between pre- and post-exposure change in MDA levels and black carbon exposure was also reported; however, no significant change in MDA levels pre- to post-exposure was noted (Wu et al., 2020a). [The Working Group judged this study as particularly informative because of the large number of paired samples (n = 81 burns and n = 39 non-burns), and the significant association between MDA and an exposure marker.]

The association between oxidative stress and career duration and age has also been investigated in firefighters from a south-eastern region of the USA. Despite overall urinary MDA and 8-oxodG levels as measured by HPLC-EDC being similar before and after a wildland firefighting shift, an increased cross-shift change in 8-oxodG levels was noted in firefighters with ≤ 2 years of experience in the role, whereas firefighters with ≥ 10 years of experience had a decrease in 8-oxodG levels (Adetona et al., 2013). Change in MDA levels from pre- to post-wildland firefighting shift was not associated with age, length of firefighter career, or $PM_{2.5}$ exposure (Adetona et al., 2013). [The Working Group noted variable exposure accumulation due to sample collection across numerous work shifts, although a large number of sample pairs (n = 94) were included in the analysis. Additionally, although age was previously reported to be associated with MDA increase, the age range was small (21–44 years), and therefore the lack of correlation was not unexpected.]

Cross-sectional analysis of blood samples from non-smoking Portuguese firefighters exposed to forest fires within the last 48 hours exhibited a level of oxidative lesions (identified using the Fpg-modified comet assay) that was 316% higher than that of the non-smoking control firefighters, and 112% higher than that of the tobacco smoke- and fire-exposed firefighters (Oliveira et al., 2020). Regarding the frequency of oxidative DNA lesions, there was a positive correlation with urinary 2-hydroxyfluorene concentration and urinary 1-OHP concentration in both exposed groups, as well as a borderline significant positive correlation with urinary 1-hydroxyphenanthrene concentration in the non-smoking exposed firefighters. [The Working Group noted that the sample size was large (n = 78 exposed, n = 93 non-exposed), and the association between oxidative stress and exposure markers was informative.]

Cross-sectional comparison of baseline blood samples revealed a higher frequency of oxidative DNA damage (detected using the Fpg-modified comet assay) in Portuguese wildland firefighters than in office workers, matched for age, gender, and smoking habits, although this difference was not significant (Abreu et al., 2017). This was despite a positive correlation reported between the level of Fpg-sensitive sites and the level of DNA damage detected using the alkaline comet assay (see Section 4.1.1). An increasing level of oxidative DNA damage with longer service was noted; however, this association was not significant. [The Working Group noted the lack of information regarding sample timing in relation to firefighting tasks but did regard the non-firefighter comparison group as a strength of the study, because of the matched characteristics.]

(iii) Employment as a firefighter

Two cross-sectional studies (Hong et al., 2000; Gündüzöz et al., 2018) assessed oxidative stress in firefighters with a history of exposure. 8-OHdG levels in the urine, as measured by ELISA, were not different in firefighters with ≥ 8 hours or < 8 hours fire exposure in the previous 5 days compared with firefighters with no exposure (Hong et al., 2000). Comparison of baseline samples from firefighters with officer controls revealed increased serum disulfide

levels and disulfide:thiol percentage ratios in firefighters, with a positive correlation between disulfide and urinary arsenic levels (Gündüzöz et al., 2018). [The Working Group noted that no information was provided regarding time since last exposure or fire types. Firefighter and control (officers) groups were well matched for age and work time; however, history of exposure for officers was not detailed.]

(iv) Exposure to heat, or mental and/or physical challenge

Three studies (Macedo et al., 2015; Park et al., 2016; McAllister et al., 2020) investigated the consequence of exercise with or without PPE on oxidative stress. In firefighter fitness tests without PPE, no changes were stimulated in blood thiol groups, total plasma antioxidant activity, SOD, CAT, GR, GSH-Px, or 8-OHdG and 8-isoprostanes (measured by ELISA) (Macedo et al., 2015). In firefighters completing a 6-week exercise training programme there were reductions in AOPP and AGE in resting plasma samples (McAllister et al., 2020). Park et al. (2016) reported that in firefighters treadmill walking (20 minutes at 25 °C) while wearing PPE and breathing apparatus there were increases in plasma levels of conjugated diene but no changes in SOD, GSH-Px, or CAT. Increased total radical-trapping antioxidant potential (TRAP) was noted, possibly indicative of increased antioxidant capacity. In addition, alterations in oxidative stress markers were not exhibited when the exercise was carried out without PPE. [The Working Group noted that the exercise modalities included in these studies, combined with the ambient environmental temperatures, may limit generalizability to firefighter suppression tasks. Heightened physical strain when wearing PPE and breathing apparatus may be associated with oxidative stress, although no statistical comparison with the group wearing regular clothing was conducted.]

Two studies (Ferguson et al., 2016; Peters et al., 2018) investigated the effect of smoke exposure on oxidative stress in controlled laboratory exposures, with participants from the general population. Participants were exposed to three conditions: filtered air (as control), 250 μg/m^3 wood smoke $PM_{2.5}$, and 500 μg/m^3 wood smoke $PM_{2.5}$, during 90 minutes of treadmill exercise. Levels of 8-isoprostane (measured by ELISA) in EBC increased 1 hour after exposure to wood smoke in comparison with filtered air, although levels were greater in filtered air immediately after exposure (Ferguson et al., 2016). Peters et al. (2018) also reported increased levels of plasma 8-isoprostane via ELISA analysis after both low (250 μg/m^3) and high (500 μg/m^3) exposures, increased MPO after both exposures, increased 3-NT after combined smoke exposure, and a decrease in the antioxidant marker UA. However, numerous markers measured (H_2O_2, EBC MPO, TEAC, LOOH, PC) did not indicate oxidative stress (Ferguson et al., 2016; Peters et al., 2018). [The Working Group noted that the physiological strain and duration of wildland exposure may not have been accurately reflected because of the selected exercise task type and duration, environmental temperature, and clothing worn.]

[The Working Group noted that robust pre/post studies in humans demonstrated correlations between exposure markers and oxidative damage, and associations between occupational firefighting exposure and oxidative stress. The study design of an additional group of studies lacked rigour, with disparities in the timing of sample collections and exposure measurements; thus, these studies were considered less informative.]

(b) Human cells in vitro

See Table 4.5.

Two studies (Park et al., 2016; Ma et al., 2020) provide in vitro assessment of oxidative stress in human cells. Isolated leukocytes from firefighters from the Republic of Korea and from

Table 4.5 End-points relevant to oxidative stress in human cells in vitro

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
GSH, ROS as DCF	Human lung epithelial cell carcinoma, A549	Training, PM collection only Denmark, particles from wood smoke with and without presence of foam and electrical cords. Particle collection by electrostatic deposition		No change in GSH ↑ ROS from wood burn only ($P < 0.05$)	Exposure duration and PM dose	Authors used a manual arbitrary scoring scale	Ma et al. (2020)
DNA damage (comet assay)	Lymphocytes	No fire exposure (treadmill exercise in temperate environment) Republic of Korea, male volunteer firefighters, repeated measures design, treadmill exercise in PPE vs regular clothing, 25 °C at 9 METS Cells exposed to H_2O_2	12 (PPE), 12 (regular clothing)	Reduced resistance to H_2O_2-induced oxidative DNA damage 40 min post ($P < 0.001$)	Cardiovascular disease, antioxidant nutrient intake	No heat/live-fire exposure; small sample size; limited ecological validity to firefighter tasks; no details on regular clothing; no statistical comparison between PPE and regular clothing trials; limited details on assay characteristics	Park et al. (2016)

DCF, dichlorofluorescein; Fpg, formamidopyrimidine DNA glycosylase; GSH, glutathione; H_2O_2, hydrogen peroxide; MET, maximal exercise treadmill training; PM, particulate matter; PPE, personal protective equipment; ROS, reactive oxygen species.
↑, increase; ↓, decrease.

Denmark, respectively, after treadmill walking (20 minutes in 25 °C) while wearing PPE exhibited reduced resistance to H_2O_2-induced oxidative DNA damage (measured by the comet assay) immediately after exercise and 40 minutes after exercise (Park et al., 2016). [The Working Group noted that no statistical comparison with the regular clothing group was conducted, values post trials appeared similar (regular clothing, tail intensity, 84.8 ± 1.3%; PPE, tail intensity, 82.4 ± 1.1%).] Assessment of the influence of smoke particles on oxidative stress measured in the lung epithelial cell line A549 indicated that ROS levels generated after 3 hours of exposure to 100 µg/mL of PM from wooden pallet burn were 50% higher than those before exposure (Ma et al., 2020). However, exposure to particle matter from wooden pallets combined with foam mattresses and electrical cords resulted in no difference in ROS generation. GSH concentration was also unaffected by PM.

(c) Experimental systems

(i) Non-human mammals in vivo

See Table 4.6.

Two studies (Demling & LaLonde, 1990; Demling et al., 1994) used experimental systems in vivo to assess oxidative stress; both used a similar protocol in adult female sheep. Sheep exposed to a tidal volume of 5 mL/kg smoke for 20 breaths exhibited increased plasma MDA immediately after exposure; this returned to baseline 1 hour after exposure and was again elevated at 24 hours after exposure. No changes in lung lymph MDA or conjugated diene were detected. Increasing smoke exposure to 10 mL/kg resulted in increased levels of lung lymph and plasma conjugated diene and MDA after exposure. These variables returned to baseline levels in 4 hours, with plasma MDA peaking again 24 hours later. Liver tissue MDA level was also doubled after exposure at the higher dose (Demling & LaLonde, 1990). Sheep exposed to 5 mL/kg smoke for an extended duration of 48 breaths exhibited increased levels of liver tissue MDA, decreased liver tissue GSH, GSSG, and CAT, decreased lung tissue CAT and decreased kidney tissue GSH, compared with control sheep (Demling et al., 1994). [The Working Group noted that smoke was generated from dyed cotton towel burning, so this study was of limited relevance to firefighters.]

(ii) Non-human mammalian cells in vitro

In the third study in an experimental system, mouse peritoneal monocytes RAW 264.7 were exposed to smoke collected from wildland fire (Leonard et al., 2007). Increased levels of H_2O_2 and MDA were detected after exposure to ultrafine (0.042–0.24 µm) and fine (0.42–2.4 µm) PM compared with a control exposure to clean air. No differences were noted after exposure to coarse (4.2–24 µm) PM. In addition, assessment in an acellular system using DNA fragments (λ Hind III fragments) revealed DNA damage, identified by increased electrophoresis band smearing, with all three PM exposure types (ultrafine, fine, coarse) and co-treatment with H_2O_2, compared with controls. The induced DNA damage was inhibited by co-treatment with sodium formate, a hydroxyl radical scavenger, and the metal chelator deferoxamine. [The Working Group noted that the inhibitor experiments indicated that a transition metal reaction with H_2O_2 was involved in the hydroxyl-generated DNA damage.]

4.1.3 Induces epigenetic alterations

See Table 4.7.

DNA methylation, post-translational histone modifications, and non-coding RNAs including microRNAs (miRNAs) were considered as indicative of epigenetic alterations. Epidemiological studies assessing DNA methylation and miRNA among firefighters were identified and reported. One of the studies also investigated epigenetic

Table 4.6 End-points relevant to oxidative stress in non-human mammalian experimental system in vivo

End-point	Species, route of exposure, doses	Tissue	Results[a]	Covariates controlled	Comments	Reference
MDA, CD	Female adult sheep, low vs high exposure exposed via intubation to smoke from burning cotton towels, 5 mL/kg smoke and 10 mL/kg smoke	Blood, lung lymph, lung, and liver tissue	*5 mL/kg smoke:* ↑ Plasma MDA post exposure and 24 h post No change in lung lymph or plasma CD or lung or liver tissue MDA *10 mL/kg smoke:* ↑ Lymph and plasma MDA and CD post exposure ↑ Plasma MDA at 24 h post ↑ Liver tissue MDA No change in lung tissue MDA	Veterinary-confirmed infection free, breath number, quantity of fuel source	Smoke from cotton towelling; 24-h study period providing time-dependent response	Demling & LaLonde (1990)
MDA, CD, CAT, GSH, GSSG	Female adult sheep, exposed vs control, exposed via intubation to smoke from burning cotton towels; 5 mL/kg smoke	Blood, airway fluid, lung lymph, lung tissue, liver tissue, kidney tissue, gut tissue	↑ Plasma MDA and CD pre to 1 h post (return to normal by 2 h) ↑ Airway fluid MDA at 12 h and 24 h compared with control No change in lung lymph CD ↓ Lung lymph MDA decreased at 4 h but returned to baseline by 18 h No change in lung tissue MDA, CD, GSH, or GSSG In lung tissue, ↓ CAT In liver tissue, ↑ MDA, ↓ GSH, GSSG, and CAT In kidney tissue, ↓ GSH No change in gut tissue for any markers	Confirmed infection free, breath number, quantity of fuel source	Some statistical data unclear; smoke from cotton towelling. 24-h study period providing time-dependent response	Demling et al. (1994)

CAT, catalase activity; CD, conjugated diene; GSH, glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; vs, versus.
[a], ↑, increase; ↓, decrease; statistical significance was defined as $P < 0.05$.

Table 4.7 End-points relevant to epigenetic alterations in exposed firefighters

End-point	Biosample, tissue, or cell type	Technical details	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significant)[a]	Covariates controlled	Comments[b]	Reference
DNA methylation								
Gene-specific DNA methylation in promoter region	Peripheral blood leukocytes	Gene specific analysis in promoter region of *GSTP1*, *DUSP22*, *RAD21*, and *IFN-γ*	Employment as firefighter USA (Ohio), Fire Service and Radiation Safety in Cincinnati, cross-sectional	18 firefighters, 20 controls	↓ *DUSP22* promoter methylation; inverse correlation with years of service	None	Small sample size; included men and women, and several current smokers; the study had in vitro data that corroborated the results for *DUSP22* Exposure assessment: adequate for primary hypothesis of higher biomarker levels in firefighters vs controls	Ouyang et al. (2012)
EWAS, DNA methylation	Peripheral blood leukocytes	Infinium EPIC array, included 834 912 CpG sites Bonferroni-correction for EWAS Pathway analysis with IPA	Employment as firefighter USA (Arizona), Tucson Fire Department, cross-sectional	41 new recruits, 45 incumbents firefighters	Incumbent vs recruits EWAS: 4 CpG sites differentially methylated Prediction analysis: 11 CpG sites predicted group and 91 CpG sites predicted years of service among incumbent FF Pathway analysis of 443 genes annotated to 512 CpG differentially methylated between incumbent firefighters and new recruits, identified enrichment for cancer-related pathways	Age, ethnicity, BMI	All non-smoking men Exposure assessment: strong methodology using fire response records to quantify proxies for exposure duration and qualitative aspects of types of fires likely correlated with chemical composition of fumes (see also Jeong et al., 2018; and Jung et al., 2021b)	Zhou et al. (2019)

Table 4.7 (continued)

End-point	Biosample, tissue, or cell type	Technical details	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significant)[a]	Covariates controlled	Comments[b]	Reference
EWAS, DNA methylation	Peripheral blood leukocytes	Infinium EPIC array, included 740 842 CpG sites FDR $q < 0.05$ for significance in EWAS Pathway analysis with missMethyl	Employment as firefighter USA, firefighters from 3 states; cross-sectional	194	Hispanic firefighters vs non-Hispanic firefighters EWAS: 54 CpG sites with lower methylation and 22 with higher methylation Pathway analysis: not significant	Gender, age, batch, blood cell proportions; sensitivity analyses with smoking and years firefighting	Comparison was meant to show ethnicity difference (only Hispanic and non-Hispanic White included) and not effect from firefighting Exposure assessment: no information on individual exposure histories	Goodrich et al. (2021b)
EWAS, DNA methylation Epigenetic age biomarkers	Peripheral blood leukocytes	Infinium EPIC array, included 740 842 CpG sites $P < 9 \times 10^{-8}$ for significance DMR analysis with DMRcate Pathway analysis with missMethyl Assessed 7 epigenetic clocks	Employment as firefighter USA, firefighters from 3 states, cross-sectional	197 firefighters	EWAS: 5 CpG sites associated with serum concentrations of 1 PFAS each DMR analysis: 3 PFAS associated with DMRs Pathway analysis: results from 3 PFAS enriched in pathways including lipid transport, immune function, cell movement Epigenetic clocks: 3 PFAS associated with ↑ epigenetic age biomarkers	Age, gender, race, Hispanic ethnicity, blood cell proportions, batch	Focus on specific PFAS chemicals Exposure assessment: no unexposed controls; range of serum PFAS concentrations but source undetermined; 9 PFAS measured in serum; other exposures not assessed	Goodrich et al. (2021a)

Table 4.7 (continued)

End-point	Biosample, tissue, or cell type	Technical details	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significant)[a]	Covariates controlled	Comments[b]	Reference
EWAS, DNA methylation	Peripheral blood leukocytes	Infinium EPIC array, included 759 346 CpG sites; FDR $q < 0.05$ for significance; pathway analysis with IPA	Employment as firefighter USA (Arizona), Tucson Fire Department, pre/post	50 new recruits before training and 20–37 months later	EWAS: 680 CpG sites changed over time (292 ↑ and 388 ↓) including 60 with ≥ 5% difference; 140 of these loci associated with number of fire-runs and time spent at fires Pathway analysis: enrichment in 9 canonical pathways and 27 disease categories including 14 cancer-related	Hispanic ethnicity, estimated smoking pack years, batch, cell type proportions	98% men Exposure assessment: strong methodology using fire response records to quantify proxies for cumulative exposure including number of fire-runs and total fire-hours; limiting study to new recruits also improved accuracy of exposure estimates	Goodrich et al. (2022)
EWAS, DNA methylation	Peripheral blood leukocytes	Infinium 450K array, included 375 223 CpG sites FDR $q < 0.05$ Pathway analysis with missMethyl	Exposure index based on time, location, and tasks of WTC response USA (New York), WTC General Responder Cohort, cross-sectional	185 responders; 69 in low and 116 in high exposure groups	EWAS: no changes Pathway analysis: 21 enriched gene-sets among top 500 CpG sites between low and high, including 7 cancer-related pathways	Age, race, smoking status, blood cell proportions	Follow-up 10 yr post-WTC event with no adjustment for exposures in between; no unexposed controls Exposure assessment: well-developed index of exposure including all available detailed self-reported information on duration of exposure and exposure-related tasks, PPE, etc.	Kuan et al. (2019)

Table 4.7 (continued)

End-point	Biosample, tissue, or cell type	Technical details	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significant)[a]	Covariates controlled	Comments[b]	Reference
Somatic mutations								
Somatic mutations in epigenetic driver genes	Peripheral blood	Deep targeted sequencing of 237 genes frequently mutated in haematological malignancies	Presence as firefighter at WTC event USA (New York), FDNY First Responders Study, cross-sectional	481 WTC responders (429 firefighters, 52 EMS) and 255 current firefighters (non-responders)	Most highly mutated genes in WTC compared with non-WTC firefighters were epigenetic regulators, *TET2* and *DNMT3A* Non-synonymous mutations in *DNMT3A*, *TET2*, and *IDH2* reported in both groups	Age, race, ethnicity, sex, smoking	Follow-up 12–14 yr post WTC event for first responders; no non-firefighter control group (see Section 4.1.1 for additional results) Exposure assessment: exposure contrast was qualitative, WTC vs "normal" firefighter exposures	Jasra et al. (2022)
microRNA expression								
miRNA expression	Peripheral blood	Blood preserved in tempus RNA tubes nCounter v3 Human miRNA expression panel, 821 miRNAs Bonferroni correction miEAA for enrichment analysis	Employment as firefighter USA (Arizona), Tucson Fire Department, cross-sectional	52 incumbent firefighters, 45 new recruits before live-fire training	Incumbents vs new recruits miRNA: 6 decreased expression and 3 increased expression (fold-change, 1.5) Enrichment analysis: targets of top miRNAs enriched for stem cells, inflammation, and cancers (melanoma, Burkitt lymphoma)	Age, BMI, ethnicity, only non-smokers included	All White men; incumbent group ~14 yr older, and 2 of the 9 significant miRNAs were also associated with age Exposure assessment: qualitative exposure assignment based on employment records; enhanced validity from comparing incumbent vs recruit firefighters	Jeong et al. (2018)

Table 4.7 (continued)

End-point	Biosample, tissue, or cell type	Technical details	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significant)[a]	Covariates controlled	Comments[b]	Reference
miRNA expression	Peripheral blood	Blood preserved in tempus RNA tubes; nCounter v3 Human miRNA expression panel, 799 miRNAs; used Bonferroni correction	Employment as firefighter USA (Arizona), Tucson Fire Department pre/post	52 new recruits before training and 20–37 months later	3 miRNA replicated from Jeong et al. (2018) Full array: 5 decreased expression and 4 increased expression in association with employment duration	Age, BMI, ethnicity, batch effects (also adjusted for time since more recent fire in some analyses)	A priori marker analysis (from Jeong et al., 2018); season of sampling was potential confounder; all white men Exposure assessment: strong methodology using fire response records to quantify proxies for exposure duration and qualitative aspects of types of fires likely correlated with chemical composition of fumes (see also Jeong et al., 2018; and Goodrich et al., 2022)	Jung et al. (2021b)

AZ, Arizona; CpG, cytosine-phosphate-guanine; DMR, differentially methylated region; EMS, emergency medical service workers; EWAS, epigenome-wide association study; epigenetic age indicators, DNA methylation-based estimators of epigenetic (biological) ageing called IEAA, EEAA, Horvath, Hannum, PhenoAge, Skin-Blood, and GrimAge; IPA, Ingenuity Pathway Analysis software; miRNA, microRNAs; PAHs, polycyclic aromatic hydrocarbons; PFAS, per- and polyfluoroalkyl substances; PFNA, perfluorononanoic acid; *q*-value, *P* value adjusted for multiple comparisons via the Benjamini-Hochberg false discovery rate (FDR) method; WTC, World Trade Center.

[a] Only statistically significant result(s) reported at appropriate *P* value cut-off used by the study (either $P < 0.05$ or adjusted for multiple hypothesis testing); "no changes" means no statistically significant results reported for any end-points of interest.

[b] Factors to be considered for study quality included the methodology, design, reporting, and quality of the exposure assessment.

alterations in vitro. The association between occupation as a firefighter and alterations in DNA methylation or in miRNA expression was investigated (Ouyang et al., 2012; Jeong et al., 2018; Kuan et al., 2019; Zhou et al., 2019; Goodrich et al., 2021a, b, 2022; Jung et al., 2021b). All studies investigated DNA methylation or miRNA in peripheral blood samples. All except one study focused on employment as a municipal firefighter, and one followed up first responders to a catastrophic event, the WTC disaster. One study reported mutations in key epigenetic regulator genes in first responders to the WTC disaster and non-WTC firefighters (Jasra et al., 2022). There were no studies investigating potential epigenetic alterations in wildland firefighters or induced by specific challenges (i.e. mental or physical, including heat). Considering the availability of data, the studies reported below are grouped by end-point.

(a) DNA methylation

Alteration in DNA methylation after occupational exposure as a firefighter was investigated in different study types. Two studies followed-up municipal firefighters (Goodrich et al., 2022) or first responders to the WTC disaster (Kuan et al., 2019); two were cross-sectional studies of incumbent firefighters (Goodrich et al., 2021a, b); and two studies compared incumbent firefighters with new recruits or non-firefighter controls (Ouyang et al., 2012; Zhou et al., 2019). In five studies (Kuan et al., 2019; Zhou et al., 2019; Goodrich et al., 2021a, b, 2022), the authors explored an epigenome-wide association study (EWAS) of DNA methylation using high-dimensional DNA methylation arrays (the Illumina Infinium 450K or EPIC arrays), which provide data at thousands of loci (called CpG sites) throughout the genome. One study employed a candidate gene approach (Ouyang et al., 2012).

Goodrich et al. (2022) sampled blood (peripheral blood leukocytes) from 50 recruits in the USA before live-fire training and again approximately 2 years later. When comparing DNA methylation data, 680 CpG sites were significantly differentially methylated (388 CpG sites had lower and 292 had greater methylation at follow-up). [The Working Group noted that associations in either direction could be important since implications for gene regulation are dependent on the genomic context.] Among these loci, 140 exhibited a significant linear association with number of fire-runs and/or time spent at fires, suggesting a dose–response with cumulative fireground exposures (see Sections 1.4.1 and 1.5.1 for chemical agents that have been observed at the fireground in other studies). Enriched gene sets among these loci included pathways relevant to carcinogenesis and tumorigenesis. [The Working Group noted that enrichment in some of these pathways, namely, molecular mechanisms of cancer, colorectal and gastrointestinal cancers, overlapped with that in other studies in the present section (Goodrich et al., 2021a, b).] [The Working Group deemed this an informative study because of the pre/post design, with repeat measures taken 2 years later. Collection of proxies for cumulative exposure, including number of fire-runs and total fire exposure time, was a strength. In addition, the results indicated persistent and cumulative DNA methylation alterations in loci annotated to cancer-related genes.]

Other DNA methylation studies provided supportive data for the influence of firefighting on DNA methylation. Zhou et al. (2019) compared DNA methylation data from blood samples (peripheral blood leukocytes) of 45 incumbent and 41 new-recruit firefighters from Arizona, USA, all non-smoking men. Methylation at four CpG sites was statistically significantly associated with firefighting, with at least a 0.5-fold difference between the two groups. In prediction analyses, methylation in 11 CpG sites predicted whether a participant belonged to the incumbent or new-recruit group, and methylation in 91 CpG sites predicted years of service among incumbent

firefighters. Pathway analysis of the most differentially methylated CpG sites identified a significant enrichment of genes in pathways relevant to tumorigenesis and tumour physiology, including sirtuin signalling, molecular mechanisms of cancer, p53 signalling, AMP-activated protein kinase (AMPK) signalling, and enriched disease pathways: abdominal cancer, colon tumours, skin cancer, and lung tumours/cancers. Goodrich et al. (2021a, b) conducted cross-sectional EWAS using blood samples from approximately 200 municipal firefighters from the USA, investigating differences in DNA methylation by ethnicity (Goodrich et al., 2021b) and by serum concentrations of PFAS (Goodrich et al., 2021a), chemicals that firefighters may be exposed to (see Section 1.5.1(b)). Of the nine PFAS measured in serum, six were detected in > 70% of participants (Goodrich et al., 2021a). When examining associations between the six PFAS and all methylated loci on the array, three PFAS (linear perfluorooctanesulfonic acid, *n*-PFOS; perfluorononanoic acid, PFNA; and perfluorodecanoic acid, PFDA) were significantly associated with DNA methylation at specific loci and multisite regions. In pathway analysis of the top loci associated with *n*-PFOS, PFNA, and PFDA ranked by raw *P* value, significantly enriched gene sets included hippo signalling, and functions related to lipid transport, ion transport, cell motility, and circadian entrainment.

Epigenetic age can be estimated from DNA methylation using data from well-validated and widely replicated CpG sites that change with chronological age. Accelerated epigenetic age has been associated with risk of cancer and mortality from cancer, including when it is measured in the blood (Perna et al., 2016). When evaluating the association between serum PFAS and seven indicators of epigenetic age in blood leukocytes, perfluorohexanesulfonic acid (PFHxS), linear perfluorooctanoic acid (*n*-PFOA), and the sum of perfluoromethylheptanesulfonic acid isomers (Sm-PFOS) were each associated with accelerated epigenetic age in multiple indicators. In contrast, PFDA and perfluoroundecanoic acid (PFUnDA) were inversely associated with one indicator. [The Working Group noted that the limitations of the study by Goodrich et al. (2021a) included the inability to identify whether the source of exposure was occupational or environmental, the cross-sectional nature of the study, and the lack of other fireground exposures measured.] [The Working Group also reviewed Goodrich et al. (2021b) but deemed it to be uninformative, because it was not investigating the impact of exposure from firefighting since all participants were incumbent municipal firefighters. Results focused on differences in DNA methylation between ethnicity groups, and they may be important when considering interindividual susceptibility to cancer in the fire service.]

Ouyang et al. (2012) conducted a study in Ohio, USA, using blood samples from 18 firefighters and 20 controls (non-firefighters) using a hypothesis-driven approach. DNA methylation was quantified at the promoter region of four genes that had been previously associated with combustion by-products or smoking in other populations. *DUSP22* promoter methylation was found to be significantly lower among firefighters than among non-firefighter controls and was inversely correlated with years of service among the firefighters but not with age in the controls. [The Working Group noted the relatively small sample size. The strengths of this study were the controlled variables and the gene selection. Moreover, the Working Group noted that the gene *DUSP22* has been related to inflammation and tumour suppressor activities in several cancers (Lin et al., 2019).] Ouyang et al. (2012) also conducted an in vitro study to build upon the epidemiological results, testing whether B[*a*]P – a combustion by-product classified in IARC Group 1, *carcinogenic to humans* – reduces promoter DNA methylation at *DUSP22* and increases its expression. Human prostate epithelial cells (NPrEC) and human T-lymphocytes

(Jurkat cells) were treated for 2 weeks with either B[*a*]P (0.1, 1, or 10 nM) or a control. Treatment was associated with a dose-dependent decrease in promoter-region DNA methylation and subsequent increase in the expression of *DUSP22*.

Kuan et al. (2019) evaluated first responders (firefighters and other responders) to the WTC disaster at multiple time-points post-exposure. DNA methylation analysis was conducted > 10 years later in blood samples from male responders who were in the top or bottom 10% of exposure according to percentiles of exposure ranking indices ($n = 116$ and $n = 69$, respectively). Exposure rank was not significantly associated with DNA methylation at any individual CpG sites at a *P* value cut-off adjusted for multiple testing. A gene-set enrichment analysis was conducted on the top 500 differentially methylated CpG sites by raw *P* value. The 21 significantly enriched gene sets included broad pathways related to cancer (i.e. "pathways in cancer" and "choline metabolism in cancer"), and other pathways relevant to tumorigenesis (i.e. "MAPK signalling"). [The Working Group noted that the limitations of this study included no adjustment for exposures in the interim (i.e. work as a firefighter after 11 September 2001), the unique exposure of WTC firefighters that may not be generalizable to other firefighters, and inclusion of primarily White male participants.]

Jasra et al. (2022) (study fully described in Section 4.1.1) reported finding in blood samples of WTC responders an increase in somatic mutations in two genes (*DNMT3A* and *TET2*) that encode epigenetic drivers – an enzyme that methylates DNA and one that is involved in active demethylation, respectively. WTC responders had more mutations overall in the blood than did firefighters who were not at the WTC. *DNMT3A* and *TET2* were the most frequently mutated genes in blood samples from WTC first-responders. Both groups had non-synonymous somatic mutations in *DNMT3A*, *TET2*, and another epigenetic regulator (*IDH2*). [The Working Group noted that these data suggested a potential mechanism for broad DNA methylation alterations in either type of firefighter. Mutation in these genes were observed with ageing (Buscarlet et al., 2017). The Working Group noted that this study lacked a non-firefighter control group.]

[The Working Group noted that collectively the above studies showed alterations in DNA methylation associated with firefighting, including alterations that persist after exposure. Several tumorigenesis- and cancer-related gene pathways were common and significantly enriched in at least two studies, including hippo signalling, circadian entrainment, AMPK signalling, general molecular mechanisms of cancer, and colorectal and gastrointestinal cancer pathways. Although these data were only available in the blood, they showed persistent alterations in DNA methylation induced by firefighting.]

(b) microRNA

Two studies in the same source population from Arizona, USA, examined associations between miRNA expression and employment as a municipal firefighter. Jeong et al. (2018) conducted a comparison of 52 non-smoking, male incumbent and 45 new-recruit firefighters, the same population described in (Zhou et al., 2019). Six miRNAs were significantly downregulated (*miR-1260a*, *miR-548h-5p*, *miR-145-5p*, *miR-331-3p*, and *miR-181a-5p*) and three were upregulated (*miR-5010-3p*, *miR-374a-5p*, and *miR-486-3p*) in incumbents compared with new recruits. [The Working Group noted that the six downregulated miRNAs have tumour suppressor functions (Epis et al., 2009; Hu et al., 2014; Ma et al., 2015; Ozen et al., 2015; Zhao et al., 2016), and two of the upregulated miRNAs (*miR-374a-5p*, *miR-486-3p*) have oncogenic properties in e.g. colorectal and oesophageal cancers (Mosakhani et al., 2012; Wang et al., 2015).] In enrichment analysis, 234 differentially expressed miRNAs were significantly associated with stem cells and significantly enriched in pathways related to

inflammation, cell adhesion-related functions, general carcinoma, Burkitt lymphoma, and melanoma.

Jung et al. (2021b) conducted a follow-up study with the same new recruits (n = 52) and re-evaluated miRNA expression 20–37 months later. The nine miRNAs identified in the cross-sectional study by Jeong et al. (2018) were compared at baseline and follow-up; three miRNAs related to cancer replicated in the same direction and were also significantly associated with employment duration: *miR-1260a* (a tumour suppressor), and *miR-5010-3p* and *miR-486-3p* (linked to cancer promotion). In the discovery full-array approach, nine additional miRNAs were identified that were significantly associated with employment duration when adjusting for structure and/or all fire-runs or fire-hours. These included four downregulated tumour suppressors (*miR-422a*, *miR-26a-5p*, *miR-92a-3p*, and *let-7f-5p*) and four upregulated oncogenes (*miR-548a-3p*, *miR-556-3p*, *miR-548ad-3p*, and *miR-525-3p*). [The Working Group considered that the strength of the study was the pre/post design and assessment of proxies for chronic and acute sources of fireground exposure, including consideration of time spent at structure fires only and all fires that the workers responded to. Replication of results across two studies was also a strength. Limitations included the small sample size, which was underpowered to detect all true associations. Mutual adjustment for employment duration and cumulative fireground responses might have attenuated the effect reported here.]

4.1.4 Induces chronic inflammation

See Table 4.8.

Alterations in inflammatory markers, such as C-reactive protein, cytokines, interleukins IL-6, IL-8, and IL-10, or tumour necrosis factor alpha (TNFα), and lung function parameters, such as the forced expiratory volume (FEV) were among the end-points considered relevant to the key characteristic "induces chronic inflammation" and reported at the beginning of the present section (Section 4.1.4(a)). Symptoms of lung dysfunction and bronchial hyperreactivity, although not directly linked to the key characteristic-associated end-points, were also considered relevant to describe the mechanistic evidence in the context of occupational exposure as a firefighter; these were also reviewed and reported at the end of the present section (Section 4.1.4(b)).

(a) Exposed humans

(i) Structure fires

Eight papers available to the Working Group reported findings from structure fires, or exposure to structure training fires. All the studies reported significant changes in markers of inflammation (e.g. various interleukins, fibrinogen, P-selectin, Club cell secretory protein (CC16; alias Clara cell protein), C-reactive protein, etc.) after fire exposure. [The Working Group noted that the strength of these papers lies in the study designs, with most papers reporting results from pre/post-fire exposure (Burgess et al., 2001, 2002; Cordeiro et al., 2021), or pre/post trial studies (Watt et al., 2016; Kim et al., 2018; Smith et al., 2019; Watkins et al., 2019a). One cross-sectional study was also included in this exposure type (Gaughan et al., 2014b).]

Several studies reported significant increases in interleukin-6 (IL-6) after exposure to live-fire structure training exercises (Kim et al., 2018; Smith et al., 2019; Watkins et al., 2019a), with Watkins et al. also reporting significant leukocytosis. IL-6 concentrations were significantly higher in fire service instructors with greater exposure to live fire because of involvement in training exercises (Watkins et al., 2019a). There was also evidence that IL-6 and fibrinogen remained significantly elevated in fire instructors 24 hours after exposure (Kim et al., 2018). [The Working Group noted that increase in fibrinogen was part of the inflammatory response.]

Table 4.8 End-points relevant to chronic inflammation in exposed firefighters

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
Structure fires							
FVC, FEV_1 (lung function) CC16 SP-A	Serum	Structure fire USA (Arizona), pre/post	51 pre/post samples 25 from Tucson, 26 from Phoenix	At Phoenix, ↓ lung function, ↑ CC16 ↑ SP-A ($P < 0.01$) At Tucson, ↑ CC16 ($P < 0.01$); no changes in SP-A and lung function	Baseline FEV_1, ever smoking, age, gender, race	No smoke exposure in 24 h before testing; participants asked to participate in overhaul only (where possible) and avoid prior entry/ventilation or extinguishing where possible; no difference between groups at baseline At Tucson, no SCBA used for overhaul; at Phoenix O_2 tanks removed, but facepieces/cartridge respirators remained; noting differing use of SCBA separate analyses were completed Exposure assessment: appropriate assessment of personal shift exposure measures in analysis and firefighting was appropriately evaluated as exposure in the pre/post design	Burgess et al. (2001)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
IL-10 IL-8 TNFα Mean FVC (lung function) CC16 SP-A	Sputum Serum	Structure fire USA (Arizona), pre/post	17 male firefighters	↓ Sputum IL-10 ($P = 0.02$); no changes observed in IL-8 or TNFα ↓ Mean FVC with smoke exposure ($P = 0.02$). ↑ CC16 ($P < 0.01$); ↑ SP-A ($P = 0.03$) indicated lung permeability after smoke exposure		Well designed; blood, pulmonary function data, and induced sputum were measured at baseline, and 1 h after overhaul Exposure assessment: engagement in smoke exposure during overhaul appropriately tested as exposure in the pre/post design; inclusion of sufficiently exposure firefighters (≥ 25 min of exposure	Burgess et al. (2002)
IL-2 IL-8 IL-10 IL-12 CC16 FVC, FEV_1, FEF25–75 (lung function)	Nasal lavage fluid (for cytokines) Sputum	Structure fire [firefighter training course] Sao Paulo, Brazil, volunteer firefighters, pre/post	37:0	↑ IL-8; ↑ IL-10; ↑ IL-2; ↑ ratio of IL-12p40:IL-12p70 ($P < 0.05$) ↓ IL-2 wk 1 to wk 4 ($P < 0.05$) ↑ CC16 ($P = 0.011$) at wk 4 vs wk 1 No changes in lung function. Significant alterations in respiratory rate, heart rate and O_2 saturation after simulation		Samples taken pre/post fire exposure, and 4 wk post exposure; 2 cohorts, no statistical differences between physical characteristics of these groups All participants used SCBA Exposure assessment: engagement in fire training appropriately tested as exposure in the pre/post design; effects of exposure to combustion by-products and heat at the same time, effect of each cannot be disentangled	Cordeiro et al. (2021)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
hsCRP FVC, FEV_1 (lung function)	Venous blood	Structure fire USA, career members of midwestern fire department, cross-sectional	401:0	↑ hsCRP-associated ↓ lung function, after adjusting for confounding variables ($P < 0.05$)	Included in regression analysis: current smoker, history of pulmonary disease, BMI, maxMETs, resting blood pressure	Single time-point; methods not clear PPE use not reported Exposure assessment: no exposure data on participants	Gaughan et al. (2014b)
IL-6 Fibrinogen	Plasma Serum	Structure fire [live-fire simulation at training centre] Republic of Korea, pre/post trial	14 firefighting academy instructors: 7 suppression simulation, 7 control group	↑ IL-6; immediately after live-fire simulation and remained elevated after 24 h; ↑ fibrinogen after 24 h		Small sample size; no significant difference between general characteristics of groups; group exertion not clearly described; smokers vs non-smokers not evenly split between groups, 5:2; wearing PPE and SCBA Inconsistency of results reported in the article Exposure assessment: involvement in controlled hot working and smoke exposure conditions appropriately tested as exposure for the effects assessments that were done in the trial	Kim et al. (2018)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
CRP IL-6 ICAM-1 P-selectin MMP-9 TAC	Serum Plasma	Structure fire Illinois, USA, male firefighters. randomized controlled trial (RCT)	24 male firefighters across 4 conditions	↑ IL-6 ($P \leq 0.0001$); ↑ MMP-9 ($P < 0.0001$); ↑ P-selectin ($P = 0.001$) No change in CRP, or TAC and ICAM-1 detected		Well designed; 9 firefighters were obese; SCBA worn Exposure assessment: engagement in simulated firefighting appropriately tested as exposure for the effects assessments that were done in the trial in relation to supplement intervention	Smith et al. (2019)
CRP IL-6 Neutrophils Lymphocytes Monocytes Eosinophils *c*TnT	Whole blood plasma	Structure fire [structure fire training exercises] United Kingdom, fire service instructors, pre/post trial	16 fire service instructors (14 men, 2 women)	↓ CRP ($P < 0.048$). ↑ Neutrophils; ↑ lymphocytes; ↑ monocytes; ↑ IL-6; ↑ *c*TnT ($P < 0.001$) No changes in eosinophils	None reported	Fire type and PM not reported; PPE worn Exposure assessment: exposure to different fire exercises appropriately tested as exposure for the effects assessments that were done in the experiment	Watkins et al. (2019a)
IL-6 Neutrophils FVC, FEV_1 (lung function)		Structure fire [structure fire training exercises] United Kingdom, fire service instructors, pre/post trial	6 fire service instructors, 6 non-firefighter controls	Fire service instructors vs controls baseline levels: ↑ IL-6; ↑ neutrophils ↑ IL-6 in fire service instructors during heat exposure and fire instruction course time periods ↓ Lung function in fire service instructors over the 4-wk training course	Time since recent exposure, no additional operational exposures	Variation in exposure duration and roles conducted; small sample size Exposure assessment: inadequate since potential simultaneous exposure to smoke was not considered; the quantitative heat exposure measure that was collected was not used in exposure–response analysis	Watt et al. (2016)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
Wildland fires							
IL-8 IL-4 IL-13 TNFα VEGF ECP Macrophages Neutrophils Eosinophils Lymphocytes FEV_1, FVC, FEF 25–75 (lung function) BHR	Sputum Serum BALF	Wildland (forest) fire Greece, 2008 forest fires, repeated measurements	60:0; post exposure vs off-season	Sputum: ↑ neutrophils ($P = 0.035$); ↑ eosinophils ($P = 0.05$); ↑ IL-8 ($P = 0.03$); ↑ TNFα ($P = 0.04$) BALF: ↑ neutrophils ($P = 0.043$); ↑ eosinophils ($P = 0.05$) Serum: ↑ IL-8 ($P = 0.03$); ↑ TNFα ($P = 0.03$); ↑ VEGF ($P = 0.02$) No changes in sputum: IL-4; IL-13; VEGF; ECP No changes in serum: IL-4; IL-13; ECP > 10 h continuous firefighting induced a more intense systemic inflammation compared with < 10 h exposure; serum: IL-8 ($P = 0.026$), TNFα ($P = 0.027$), and VGEF ($P = 0.021$) ↓ Lung function post exposure compared with off-season No changes in BHR off-season and post-exposure		Thorough clinical assessment; 87% current smokers with history of 9 ± 5 packs/year Exposure assessment: time away from firefighting adequately assessed	Gianniou et al. (2018)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
42 inflammatory cytokines, chemokines, and growth factors FEV_1, FVC (lung function)	Plasma	Wildland (forest) fire Fort McMurray fire, Canada, 2016, repeated measurements	160 (148 men) firefighters from 2 fire services. Samples collected 19 days of the start of the fire (early sample) and again 14–18 wk later (late sample)	25/42 inflammatory markers ↓ ($P < 0.05$) from early to late samples Second component of inflammatory markers associated with ↓ lung function ($P = 0.032$) Clustered within fire service, cumulative exposure, dehydration, and time since last deployed to a fire were all related to the second principal components late cluster scores of inflammatory markers		Unbalanced samples/ time-point; differences in tasks/roles for each group; principal components analysis conducted to reduce the dimensionality of the inflammatory marker arrays and extract uncorrelated component scores Exposure assessment: measurements on exposure levels at group level, not for individual workers, so possible exposure misclassification; possible unmeasured events before or after the fire	Cherry et al. (2021)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
IL-6 IL-8 GM-CSF MCP-1 FEV_1 (lung function)	Serum Sputum	Wildland (forest) fire Canada, seasonal forest firefighters, pre/post	52:0 Before and after a day of firefighting	Serum: ↑ IL-8 ($P < 0.001$); ↑ IL-6 ($P < 0.02$); ↑ MCP-1 ($P < 0.02$). Sputum: macrophages containing phagocytosed particles and circulating band cells No changes in GM-CSF No changes in lung function		Pre/post 8-h shift samples Healthy non-smoking firefighters aged 17–60 yr were eligible Exposure assessment: although misclassification was possible with self-reported smoke intensity, carbon monoxide concentrations as surrogate for particulate matter exposure were used to confirm presence of smoke; firefighting shift appropriately tested as exposure for pre/post comparison	Swiston et al. (2008)
CRP IL-1β IL-8 SAA ICAM-1 VCAM-1	Dried blood spot	Wildland (forest) fire Savannah river site, USA, pre/post	12 firefighters (10 men, 2 women)	↑ IL-8 ($P = 0.0012$) Firefighters who lit the fires as opposed to other tasks had ↑ IL-8 ($P = 0.0186$). No changes in IL-1β, CRP, SAA, ICAM-1, VCAM-1	Work shift exposure to $PM_{2.5}$ and CO_2, gender, number of burns before sampling, work task, age, BMI, illness status, or allergies	Exposure assessment: appropriate personal shift $PM_{2.5}$ exposure measure; firefighting was appropriately evaluated as exposure in the pre/post design	Hejl et al. (2013)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
IL-1β IL-2 IL-4 IL-5 IL-6 IL-7 IL-8 IL-10 IL-12p70 IL-13 IFN-γ TNFα GM-CSF	Plasma	Wildland (forest) fire Australia, pre/post	12 male CFA firefighters 2 consecutive days, 3 timepoints (pre-, post, and 2 h post-shift)	Significant change in IL-6 after exposure (within same days) and between days (repeated exposure over days) ($P = 0.037$) *Within-day*: ↑ IL-1β; ↓ IL-5, ↑ IL-7, ↓ IL-10, and ↓ TNFα (all $P < 0.01$) IL-1β and IL-7 returned towards baseline after end of shift. ↓ IL-5; ↓ IL-10 and ↓ TNFα 2 h post-shift compared with baseline ($P < 0.01$) *Between days:* Significant effect of performing repeated shifts on several inflammatory cytokines. IL-1β ($P = 0.005$), IL-7 ($P = 0.004$), IL-4 ($P = 0.048$), IL-6 ($P = 0.036$), IL-8 ($P = 0.045$), and IL-13 ($P = 0.05$) all presented with an attenuated response across the course of the second day		Standard fire-retardant personal protective clothing was worn throughout the shift as per agency guidelines, but no respiratory PPE/SCBA was used Exposure assessment: all workers were exposed; no differentiation between workers; no individual data on tasks performed at site taken into account; possible unmeasured events before or after shift	Main et al. (2013)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
13-plex cytometric bead array kit (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IFN-γ, GM-CSF, and TNFα)	Plasma	Wildland (forest) fire (suppression activities after Black Saturday natural disaster) Australia, pre/post 12-h shift of wildfire suppression, pre/post	38 male CFA volunteer firefighters; 0 controls	↑ IL-6 ($P = 0.003$); ↑ IL-8 ($P = 0.017$); ↓ IL-10 ($P = 0.021$) No changes in any other biomarker		High-sensitivity assay used Standard fire-retardant personal protective clothing was worn throughout the shift as per agency guidelines, but no respiratory PPE/SCBA Exposure assessment: all workers were exposed; no differentiation between workers; no individual data on tasks performed at site taken into account; possible unmeasured events before or after shift	Main et al. (2020)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
CRP IL-6 IL-8 sICAM-1	EBC	USA, Savannah River site, pre/post shift, and next morning, pre/post	12 healthy wildland firefighters (9 men and 3 women)	No significant changes observed across the prescribed burn shifts for any of the inflammatory markers		Data collected after 7 prescribed burn shifts (burn days), as well as 3 regular work shifts (non-burn days) Small sample size; question as to feasibility of EBC for measuring inflammatory cytokines; only 3/142 EBC samples had detectable IL-6 levels Exposure assessment: firefighting appropriately used for analysis in the pre/post comparisons; no personal monitoring data was used in analysis	Wu et al. (2020b)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
Employment as a firefighter							
IL-1β IL-6 IL-8 IL-10 INF-γ TNFα FVC, FEV_1 (lung function) Serum pneumoproteins BHR	Sputum Serum	Employment as a firefighter Netherlands, repeated measurements, samples at 24 h, 1 wk and 3 months post-exposure	51:0 control 37 volunteer 8 professional [career] 6 both volunteer and professional [career]	Serum: ↑ IL-8 at 24 h ($P = 0.031$), 1 wk ($P = 0.0007$; ↑ IL-6 and ↑ IL-8 3 months after exposure ($P < 0.0001$) compared with pre-exposure Sputum: ↑ neutrophils positively associated with IL-8 ($P = 0.0023$), IL-10, ($P = 0.023$), and TNFα ($P = 0.011$) in serum within 24 h after exposure Perceived exposure was positively associated with a change in IL-8 after 1 wk ($P = 0.001$) 44% of firefighters had elevated sputum neutrophil levels (> 60%) No changes in BHR, lung function and serum pneumoprotein levels	Questionnaire assessed exposure including job history, working years, use of SCBA	Sputum was induced within 5 days of smoke exposure Exposure assessment: detailed self-reported exposure information and objective data on particle counts in induced sputum	Greven et al. (2012)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
IL-8 ECP VEGF TNFα Macrophages Neutrophils Eosinophils Lymphocytes FEV_1, FVC (lung function)	Serum Sputum BALF Bronchial biopsies (for a subgroup of 20)	Employment as a firefighter Greece, cross-sectional	63 professional [career] firefighters with 9 ± 1 yr in service; 29 trainees with 1 ± 0.1 yr; 18 healthy controls	Professionals vs trainees Sputum: ↑ eosinophils ($P < 0.05$); ↑ IL-8 ($P = 0.04$); ↑ ECP ($P = 0.02$); ↑ VEGF ($P = 0.04$); ↑ TNFα ($P = 0.02$) Serum: ↑ IL-8 ($P = 0.04$); ↑ TNFα ($P = 0.03$) BALF: ↑ eosinophils ($P < 0.05$) Trainees vs controls Serum and sputum: ↑ IL-8; ↑ TNFα Duration of the occupation in service correlated with higher number of cells in sputum and BALF, higher percentage of eosinophils, neutrophils, and lymphocytes No significant differences in lung function between groups	Comparison were adjusted for age, smoking pack-years and pre-existing diagnosed asthma	Exposure assessment: employment categories used for effects comparisons likely adequate; potential confounding of career length with age	Gianniou et al. (2016)
CRP IL-6 IL-1β Neutrophils	Venous whole blood Plasma	Employment as a firefighter United Kingdom, cross-sectional	57 firefighters; 53 fire service instructors	Fire service instructors vs firefighters ↑ Neutrophils; ↑ IL-6; ↑ IL-1β; ↑ CRP ($P < 0.05$) Multiple regression analysis revealed 18.8% of IL-6; 24.9% of IL-1β, 29.2% of CPR could be explained by the number of heat exposures/month		Fire exposures, and health complaints self-reported Exposure assessment: self-reported frequency prone to misclassification	Watkins et al. (2021)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
IL-6 IL-1β Neutrophils Eosinophils CRP	Whole blood Plasma	Employment as a firefighter United Kingdom, fire service instructors, pre/post trial	11 fire service instructors: 11 controls	↑ Neutrophils; ↑ IL-6, ↑ IL-1β, ↑ CRP, after heat exposure irrespective of group ($P < 0.05$) Fire service instructors vs controls: Resting ↑ IL-6; ↑ IL-1β ($P < 0.05$)	None reported	40 min walk test (6 W/kg) in climate chamber at 50 °C ± 1.0 °C; PPE worn Exposure assessment: number of self-reported fires may be misclassified; heat exposure was under controlled condition	Watkins et al. (2019b)
CRP FVC, FEV_1 (lung function) SAA ICAM-1 VCAM-1	Plasma	Employment as a firefighter Denmark, pre/post 24-h shift sample pairs, pre/post	22 men	↓ Lung function; ↑ VCAM-1 ($P < 0.05$) No changes in ICAM-1, SAA, and CRP IL-6 and IL-8 below LOD		Small sample size; only 3 days without work may have resulted in elevated levels of biomarkers pre-shift; the biological effective dose may not have been sufficiently large in present study to elicit expected responses Exposure assessment: firefighting was appropriately evaluated as exposure in the pre/post design; other exposure measures apparently not used in effect analysis; some logistic difficulties	Andersen et al. (2018b)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
IL-1RA Macrophages FVC, FEV_1 (lung function)	Sputum	Employment as a firefighter USA (Arizona), cross-sectional	67 firefighters (64 men, 3 women) Average service, 16.6 yr (range, 3–32 yr)	↑ IL-1RA ($P = 0.025$); ↑ macrophage count ($P = 0.002$) associated with a slower rate of FEV_1 decline	Ethnicity, sex, age, baseline FEV_1, ever-asthma, ever smoker, weight change	Participants provided ≥ 4 pulmonary function tests in 7 yr Exposure assessment: genetic polymorphism was the "exposure" of interest; self-reported occupational/ firefighting-related exposure information collected, but not used	Josyula et al. (2007)
Exposure to heat, mental or physical challenges							
PTX3	Plasma and EBC	[Wildland] wood smoke, mimicking wildland firefighter activities USA, pre/post trial	10:0 Exposed to 3 doses of wood smoke $PM_{2.5}$ (filtered-air, 250 µg/m³, and 500 µg/m³) while exercising on a treadmill	Plasma ↑ PTX3 immediately post-exposure, ($P = 0.048$) and 1 h post-exposure ($P = 0.012$) No changes in PTX3 concentration in EBC		Exposure assessment: the controlled exposure to different concentrations appropriate for the pre/post design	Ferguson et al. (2016)
Leukocytes Neutrophils TNFα IL-6 IL-10 CRP	Serum	Heat, mental, physical challenges [repeated work protocol in heat chamber (100 ± 5 °C)] Australia, purpose-built climate chamber (100 °C ± 5 °C), pre/post trial	42 urban firefighters	*Pre/post:* ↑ TNFα; ↑ IL-6 ($P < 0.05$); ↑ leukocytes; ↑ neutrophils ($P < 0.01$), *After 24 h:* ↓ TNFα; ↓ IL-6 ($P < 0.01$) No change in CRP		Exposure assessment: exposure to heat appropriately tested as exposure for the effects assessments that were done in the trial	Walker et al. (2015)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
Leukocytes TNFα CRP		Heat, mental, physical challenges [repeated work protocol in heat chamber (100 ± 5 °C)] Australia, pre/post trial	Same cohort as above	Higher baseline leukocytes observed for high body fat ($P = 0.002$) and low mean mass ($P = 0.023$) Significant lower values for TNFα with high lean mass at all time-points	None reported	Similar data set as Walker et al. (2015) Exposure assessment: exposure to heat appropriately tested as exposure for the effects assessments that were done in the trial	Walker et al. (2017)
IL-6 IL8 IL-1β TNFα IL-4 IL-10	Finger prick plasma	Heat, mental, physical challenges Australia, CFA volunteers, pre/post trial	18 controls; 17 sleep-restricted	IL-6 diurnal values above normal levels in both groups Across days: ↑ IL-6 ($P < 0.05$) Within days: ↑ IL-6; ↑ IL-4; ↓ IL-1β; ↓ TNFα; ↓ IL-8 ($P < 0.05$) IL-8 higher in firefighters who received 8 h sleep ($P < 0.05$) No changes in IL-10		Controlled study design with a control group investigating impact of restricted sleep on firefighters when performing simulated wildfire suppression tasks PPE worn but no SCBA Linear mixed models with restricted maximum likelihood Exposure assessment: longer sleep opportunity does not automatically result in more sleep; authors did present the actual hours slept, which was significantly different between groups	Wolkow et al. (2015a)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
IL-6 IL8 IL-1β TNFα IL-4 IL-10	Finger prick plasma	Heat, mental, physical challenges Australia, CFA volunteers, pre/post trial	18 controls; 17 sleep-restricted	Morning ↑ IL-6 related to ↑ cortisol ($P < 0.05$) in sleep-restricted firefighters	Age, BMI, sex	Controlled study design with a control group investigating impact of restricted sleep on firefighters when performing simulated wildfire suppression tasks; PPE worn but no SCBA; 3 days of simulated wildfire suppression tasks ± restricted sleep Exposure assessment: longer sleep opportunity does not automatically result in more sleep; authors did present the actual hours slept, which was significantly different between groups	Wolkow et al. (2015b)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
IL-6 IL8 IL-1β TNFα IL-4 IL-10	Finger prick plasma	Heat, mental, physical challenges [3 days of simulated wildfire suppression tasks ± restricted sleep] Australia, CFA volunteers, pre/post trial	Control, 18 (mild temperatures); 19 (hot temperatures)	Mild vs hot temperatures ↑ IL-4 ($P < 0.05$) Significant condition × time interaction IL-1β, which was consistently lower in hot conditions ($P = 0.011$) Significant day × time interaction for IL-1β in hot conditions, which were higher on D1 vs D3 at 06:15 ($P < 0.05$) and 11:30 ($P < 0.01$). indicating a decrease in IL-1β across days Significant fixed effect of time on IL-6, increasing across time-points ($P < 0.001$); significant day × time effect for IL-6 ($P < 0.05$) showed Il-6 increased from day 1 to 2 Fixed effect of time for TNFα ($P < 0.02$) and IL-8 ($P < 0.04$) indicating a decrease across time Morning IL-6 positively correlated with elevated cortisol ($P < 0.024$)		Controlled study design with a control group investigating impact of heat exposure on firefighters when performing simulated wildfire suppression tasks; ambient temperature for hot condition was 33 °C; PPE worn but no SCBA Exposure assessment: exposure to 2 different temperatures appropriately tested as exposure for the effects assessments that were done in the trial	Wolkow et al. (2017)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
CRP IL-6 TNFα	Whole blood Serum IL-6 and CRP Plasma TNFα	Heat, mental, physical challenge [strenuous work (physical activity) in the heat (with or without humidity to simulate impact of PPE)] Ottawa, Canada, firefighters, pre/post trial	12 older firefighters (age, 49.8 ± 1.1 yr); 12 non-firefighters age-matched (age, 51.7 ± 1.5 yr); and 6 younger firefighters (age, 26.7 ± 0.8 yr) and 6 age-matched (age, 24.8 ± 1.4 yr) non-firefighters	IL-6: showed group × time × condition effect. IL-6 significantly higher post warm/humid conditions vs warm/dry for non-firefighters ($P < 0.05$) but not firefighters; IL-6 also significantly higher in non-firefighters post warm/humid than in firefighters ($P < 0.05$) CRP: significantly decreased with time pre to post in both groups and conditions ($P < 0.05$) TNFα: no significant changes within or between groups, or over time	Age, humidity [not included as true covariates but examined within the analysis]	20 min baseline, HR monitor worn, performed 4 × 15 min cycling at 400 W (~45% of VO_{2peak}) in dry or humid conditions: 35 ± 0.1 °C and 20 ± 1.5 RH (warm/dry) vs 35 ± 0.1 °C and 60 ± 1.0 RH Exposure assessment: exposure to different humidity conditions appropriately tested as exposure for the effects assessments that were done in the trial	Wright-Beatty et al. (2014)
IL-6 Et-1 TXB_2	Plasma	Heat, mental, physical challenges [physical challenge (bike ergometer) ± dual FSTD mental challenge USA, professional [career] firefighters, pre/post trial	12 professional [career] firefighters 11.58 ± 7.52 yr experience	No differences between conditions for IL-6, Et-1 or TXB_2 Positive correlation between cortisol, IL-6, Et-1, and TXB_2 Negative correlation between IL-6 and TXB_2	NR RMANOVAs used with paired samples *t*-tests for between conditions analyses	Well controlled Exposure assessment: exposure to exercises or mental challenge appropriately tested as exposure for the effects assessments that were done in the trial	Webb et al. (2011)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
IL-2 IL-6	Plasma	Heat, mental, physical challenges USA, exercise with and without FSTD, pre/post trial	9 professional [career] male firefighters	Significant condition × time interaction for IL-2 ($P < 0.05$) NS change over time for IL-6 under either condition		Dual task challenge using computer decision-making FSTD while exercising; low workload selected to limit stimulating markers of inflammation because of prolonged high-intensity training Exposure assessment: engagement in controlled drill exercise appropriately tested as exposure for the effects assessments that were done in the trial	Huang et al. (2010a)
Catastrophic events							
WTC-lung injury CRP FEV_1 (lung function) Apo-AII MIP-4 sVCAM MPO	Serum	WTC firefighting, WTC-exposed firefighters, nested case–control study	124/171 subcohort controls, 68/100 WTC-LI (lung injury) resistant cases, and 66/100 WTC-LI susceptible	WTC-LI susceptible cases had higher Apo-AII, CRP, and MIP-4 Resistant WTC-LI cases had significantly higher sVCAM and lower MPO			Weiden et al. (2013)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
Macrophages Neutrophils Lymphocytes Eosinophils MMP-9	Induced sputum	WTC firefighting, cross-sectional study	39 highly exposed firefighters (FDNY-FF); Control groups of 12 Tel-Aviv firefighters (TA-FF) and 8 Israeli health care workers not exposed to WTC dust	FDNY-FF vs TA-FF vs controls ≥ 10 days work at WTC associated with significantly higher percentage of neutrophils ($P = 0.046$); and eosinophils ($P = 0.038$) Trend for higher MMP-9 in FDNY-FF vs TA-FF Both firefighter groups significantly higher than control ($P = 0.0001$)	Current or post tobacco smokers were excluded	Unbalanced sample sizes; single time-point, 10 months post exposure; non-parametric analyses used Exposure assessment: it did not account for potentially confounding exposure in the intervening period between exposure of interest and measurement of effects; self-reported/ qualitative exposure among exposed groups used in analysis	Fireman et al. (2004)
FEV_1 (lung function) Leukocytes	Whole blood	WTC-exposed firefighters, pre/post	9434 for FEV trajectory analysis 2103 for secondary airflow limitation analysis	Higher blood eosinophil and neutrophil concentrations each associated with accelerated FEV_1 decline after adjustment for covariates (OR, 1.10 per 100 eosinophils/mL; 95% CI, 1.05–1.15; and OR, 1.10 per 1000 neutrophils/mL; 95% CI, 1.05–1.15, respectively)		Individuals experiencing accelerated FEV_1 decline were more likely to have incident airflow limitation	Zeig-Owens et al. (2018)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
FEV_1 (lung function) MMP-3 MMP-12 MMP-1, -2, -7,-8, -9, and -13	Serum	WTC firefighting, nested case–control study	70 with WTC-L1 (lung injury); 123 controls from initial cohort of 1720	↓ MPP-3; ↓ MMP-12 ($P < 0.05$) Elevated MMP-3 and MMP-12 within 200 days of WTC exposure showed reduced odds of developing WTC-LI by 73% and 54% respectively Elevated MMP-1 and -8 but not predictive of lung injury No changes in MMP-2, MMP-7, MMP-9, and MMP-13 expressions			Kwon et al. (2013)
FEV_1 (lung function) MMP-2 TIMP-1	Serum	WTC firefighting, nested case–control study	Baseline cohort, 801 (never smokers) Resistant cases, 100; 77 with serum (recovered FEV_1 quicker) Cohort controls, 171; 137 with serum	Significant difference in lung function between cohort controls and those that were more resistant to persistent lung function decline ($P < 0.001$) From chest CT imaging: 14% of resistance cases had bronchial wall thickening, whereas 35% of the controls had evidence of airway inflammation ($P < 0.03$) MMP/TIMP balance reflects independent pathways to airway injury and repair Elevated TIMP-1 and MMP-2 predicts recovery of lung function Elevated MMP-1 reduces odds of recovery, years after WTC exposure	Pre-9/11 FEV_1, BMI, age		Nolan et al. (2014)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
Chronic rhinosinusitis Sinus polyps IL-6 IL-8 TNFα Neutrophils FEV_1 (lung function) PMN	Serum	WTC firefighting Nested case–cohort study	179 study patients: 76 developed chronic rhinosinusitis; 62 were medically managed and 14 were refractory to medical management (≥ 3 months) and elected to have surgery	IL-8; TNFα and PMN count significant predictors ($P < 0.05$) of sinus disease severity Increasing IL-6, IL-8, GRO and neutrophil concentrations reduced risk of chronic rhinosinusitis progression; increased TNFα, increased risk of progression No significant differences in spirometric parameters including FEV_1 and FEV_1/FVC in cases vs controls Increase in IL-6 decreased the odds of abnormal FEV_1	Biomarkers used as continuous covariates in logistic regression models	6 months post exposure to 9/11; presence of sinus polyps indicative of chronic inflammation Exposure assessment: self-reported exposure, which will be accurate for the time of arrival; no individual data on tasks performed at WTC taken into account; possible unmeasured events before or after WTC exposure	Cho et al. (2014)
COPD Asthma Cytokines Eosinophils	Serum	WTC firefighting, repeated measurements	Subgroup of 215 from 2137 WTC-exposed	Eosinophil concentration ≥ 300 cells/μL was associated with increased risk of asthma/COPD overlap, but not with either in isolation IL-4 predicted asthma/COPD overlap; greater IL-21 concentration associated with isolated-asthma and isolated-COPD	Age, race, smoking, WTC exposure, first post-9/11 FEV_1/FVC ratio, and BMI	Reported results of regression models and 95% CI Exposure assessment: self-reported exposure, which will be accurate for the time of arrival; no individual data on tasks performed at WTC taken into account; possible unmeasured events before or after WTC exposure	Singh et al. (2018)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
FEV_1 (lung function) GM-CSF IP-10 MDC	Serum	WTC firefighting, nested case–control cohort study	801 baseline cohort (never smokers) 70 cases of airflow obstruction 124 controls	Lung function ↓ in 12% of cases and ↑ in 3% of controls Elevated GM-CSF and MDC levels associated with increased risk of airflow obstruction in subsequent years	BMI, age, PMN	Cases of airflow obstruction defined as FEV_1 < the lower limit of normal (LLN)	Nolan et al. (2012)
FEV_1 (lung function) LPA Apo-A1 PMN	Serum	WTC firefighting, nested case–control study	801 baseline cohort: 62 cases and 111 controls	PMN count included in multivariable logistic model to predict decline in lung function and likelihood of developing WTC-lung injury		Exposure assessment: self-reported exposure, which will be accurate for the time of arrival; no individual data on tasks performed at WTC taken into account; possible unmeasured events before or after WTC exposure	Tsukiji et al. (2014)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
MCP-1 LPA Cytokines	Blood serum	WTC firefighting, first responders who had lung damage up to 16 yr after 11 September 2001, cross-sectional	15 cases with lung damage from WTC exposure, and 15 controls	↑ MCP-1 Positive correlations between LPA and membrane-bound soluble receptor for advanced glycation end-products; and positive correlations among various cytokines and chemokines; strong negative correlations between the cytokines and chemokines and several sphingolipids and omega fatty acids	Smoking	Statistically rigorous; only 1 analyte was at a higher concentration in exposed group; the other 8 analytes were at concentrations not significantly different between exposed and controls; correlation matrix of serum biomarkers in WTC-exposed first responders Exposure assessment: no information on exposures in the intervening period between exposure of interest and measurement of effects, but information used probably sufficient for research questions	Lam et al. (2020)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
Sarcoidosis	Serum	WTC-exposed firefighters with sarcoid arthritis, repeated measurements	11 reported in study From 9/60 plus 2 of ongoing monitoring	Descriptive account 1 out of 9 elevated CRP Chronic inflammatory polyarthritis appears to be an important manifestation of sarcoidosis WTC exposure		Biopsy-proven sarcoidosis; 9 by transbronchial or mediastinal biopsy, 1 by both liver and bone biopsies, and 1 by Kveim testing Exposure assessment: self-reported exposure, which will be accurate for the time of arrival; no individual data on tasks performed at WTC taken into account; possible unmeasured events before or after WTC exposure	Loupasakis et al. (2015)
Sarcoidosis	Peripheral whole blood	WTC-exposed firefighters, nested case–control study	55:100	17 allele variants of HLA and non-HLA genes were found to be associated with sarcoidosis; similarities found between genetic variants with WTC-related sarcoidosis and those reported previously in sporadic sarcoidosis cases within the general population		Specifically reporting on genetic variants associated with WTC-related sarcoidosis Exposure assessment: genotype/genetic variants was the actual "exposure" of interest in this susceptibility study of sarcoidosis among WTC firefighters; no data on tasks of airborne exposures	Cleven et al. (2019)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
Lung function process alteration and bronchial hyperreactivity							
FEV_1, FVC (lung function) Functional polymorphisms in TNFα; TGFβ1; IL-1β; IL-1RN; IL-13; and IL-8 genes	Blood or buccal cell samples for DNA analysis	Employment as a firefighter USA (Arizona), Phoenix Fire Department subset of active firefighters, cross-sectional study of subset from available medical data (1988–2003)	451 active firefighters	Interindividual variability in progressive decline in FEV_1 may be explained in part by genetic variations within genes involved in inflammatory responses	Age, race, ethnic group, smoking status, gender	Exposure assessment: "active firefighter" is a crude measure of exposure with potential for misclassification	Yucesoy et al. (2008)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
FEV_1, FVC (lung function) CC16 SP-A BHR	Serum	Employment as a firefighter Netherlands, cross-sectional study from 23 brigades	402 firefighters 356 men, 46 women (combination of 305 volunteers, 60 professional [career], 37 both)	CC16 was negatively associated with the number of fires fought in last 12 months in current non-smokers ($P = 0.04$); this grew stronger when adjusted for FEV_1 ↑ CC16 in male firefighters ($P = 0.04$), positively associated with FEV_1 and FVC ($P = 0.03$) When the analysis was stratified for atopy, a weak association ($P = 0.07$) was found between CC16 and dose response slope (% decline in FEV_1/mg inhaled methacholine); which grew stronger when adjusted for smoking ($P = 0.04$) SP-A was positively associated with exposure to fire smoke within 2 days preceding testing for those that also had respiratory symptoms ($P = 0.003$), and this became stronger when adjusted for smoking ($P = 0.0007$); the strength of this relationship increased with reduction in time between exposure and testing. (i.e. < 24 h, $P = 0.0001$; vs < 3 days $P = 0.120$)	Sex, age, atopy, BMI, diurnal variation, smoking behaviour, lung function (FEV_1 and FVC), sampling time	Large cross-sectional study, with wide range of covariates controlled for Exposure assessment: self-reported exposure is prone to bias and misclassification, particularly with regard to frequency (number of fires fought)	Greven et al. (2011a)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
FEV_1 (lung function) IL-10 genetic polymorphisms	Whole blood samples Buccal cells in mouthwash	Employment as a firefighter Arizona, USA, repeated measurements	1204 firefighters with ≥ 6 annual FEV_1 measures	↓ Lung function based on genotyping at the 1668 SNPs	Age, gender, race/ethnicity, smoking, baseline FEV_1	379 with SNP data	Burgess et al. (2004)
FEV_1, FVC (lung function) Airway responsiveness, HCT for provocation	NA	Training (smoke chamber) Singapore, recruits and professional [career] firefighters, pre/post	10 new recruits and 10 professional [career] firefighters	Airway responsiveness observed only among professional [career] firefighters after the challenge Changes in ventilatory function were seen in firefighters No changes in adjusted analyses	Age, height, length of service, time in smoke chamber, smoking pack-years, and pre-exposure level	All participants were smokers and male; results were only significant in unadjusted analyses Exposure assessment: high level, brief exposure was assured by design, but exposure intensity and composition not measured	Chia et al. (1990)
BHR FEV_1, FVC (lung function) Self-reported respiratory symptoms		Wildland (forest) firefighting Greece Forest firefighters from 2008, Repeated measures (follow-up within 1 wk of exposure and in the off-season ~3 months later)	60 with measures < 1 wk post-exposure and in the off-season	Post-exposure compared with off-season: pulmonary function effects ↑ Respiratory symptoms (wheezing, cough, expectoration, chest tightness) No differences in BHR	None	Cases serve as their own controls and may work as municipal firefighters in the off-season; all participants are male and high proportion smoke (87%) Exposure assessment: time away from firefighting adequately assessed	Gianniou et al. (2018)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
FEV_1, FVC (lung function)	NA	Wildland Portugal, active wildland firefighters, cross-sectional	209 firefighters, no controls	11.8% had obstruction. 41% of obstructed individuals were non-smokers Progressive decline in FEV_1 and FEV_1/FEV_6 with increasing length of service	None	Descriptive study; reliant on self-reported data; 85.7% not using PPE; 42.9% smokers Exposure assessment: self-reported smoke intoxication may be misclassified; duration of service will however be relatively reliable	Almeida et al. (2007)
Bronchial reactivity FEV_1, FVC (lung function) Self-reported respiratory symptoms	NA	Employment as firefighter Greece, firefighters, cross-sectional	63 professional [career] firefighters, 29 trainees with < 1 yr experience; 18 healthy controls	↑ Atopy, allergic rhinitis, cough, dyspnoea, and BHR among professional [career] firefighters (BHR, 21% compared with 3% trainees)	Age, smoking, and pre-existing asthma in some comparisons	Source of controls unspecified; all men Exposure assessment: employment categories used for effects comparisons likely adequate; potential confounding of career length with age	Gianniou et al. (2016)
FEV_1, FVC (lung function) BHR Atopy	Blood	Employment as firefighter Netherlands, 54 municipal fire brigades, cross-sectional	402 firefighters, 305 volunteers, 60 professional [career], 37 both	↑ BHR associated with the number of fires fought in the last 12 months with ($P = 0.018$), and without ($P = 0.03$) adjustments for covariates); but not associated with working years This association was stronger among atopics	Smoking, sex, atopy, age, and exposure in main job held	Self-reported smoke exposure potentially problematic Exposure assessment: self-reported exposure prone to bias and misclassification, particularly with regard to the frequency (number of fires fought)	Greven et al. (2011b)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
FEV_1, FVC (lung function) DL_{CO}	NA	Employment as firefighter USA (Washington), Seattle firefighters voluntary medical surveillance programme, repeated measures	812 firefighters with ≥ 2 yr DL_{CO}	Stable ventilatory capacity overtime was observed Overtime, DL_{CO} decline of –1.02 mL/min per mm Hg associated with year of measurement; decline of –0.006 mL/min per mm Hg associated with number of fires fought	Age, gender, race, height, prior smoke exposure	Annual measures over an 8-yr period, ≥ 2 yr DL_{CO} needed for inclusion Self-report questionnaires for exposure potentially problematic	Burgess et al. (1999)
FEV_1, FVC (lung function) Obstruction	NA	Employment as firefighter USA (Connecticut), cross-sectional	22 non-smoking firefighters; 31 smoking firefighters	35% of smokers and 13% of non-smokers had airway obstruction. In non-smoking group, obstruction only present in firefighters with > 25 yr-experience	Smoking, years of firefighting, age	Self-reported respiratory and occupational questionnaire	Loke et al. (1980)
Respiratory symptoms	NA	Employment as firefighter Netherlands, 54 municipal fire brigades, cross-sectional	1330 active firefighters Random sample of 2711 from Dutch population	Strong association found between self-reported inhalation incident and presence of respiratory symptoms (i.e. atopy, asthma, BHR-like symptoms)	Smoking, sex, atopy, age	Self-reported smoke exposure potentially problematic Exposure assessment: self-reported exposure prone to bias and misclassification, particularly with regard to the frequency (number of fires fought)	Greven et al. (2011c)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
FEV_1, FVC (lung function) Respiratory symptoms (upper and lower)	NA	WTC firefighting USA (New York), WTC Worker and Volunteer Medical Screening, pre/post (screened between 1 and 2.5 yr post-9/11)	9442 first responders	New or worsened upper and lower respiratory symptoms reported after 9/11 (compared with before), highest among workers that arrived on 9/11 and worked in the dust cloud At time of follow-up, 20.5% had low FVC compared with 4.4% expected in the general population	None; categorized participants by date of arrival and exposure to dust cloud	Eligible participants included any worker (firefighters but also others) in search/rescue/clean-up for ≥ 80 h or working with human remains examinations for ≥ 25 h	Herbert et al. (2006)
Bronchial reactivity (MCT) FEV_1, FVC (lung function)	NA	WTC firefighting USA (New York), WTC firefighters, pre/post (with follow-ups pre-9/11 and 2 post-9/11, 2 yr and > 10 yr after)	173 firefighters with pre-9/11 health data and 2 post-9/11 MCT measures	16% and 25% had BHR at the first and second follow-ups; BHR at follow-up associated with ↓ FEV_1 rate (15.39 mL/yr)	Age, abnormal lung function at baseline, smoking	MCT method differed at the first and second post-9/11 visits; the first may be overestimated; selection bias possible; all men, 95% White Exposure assessment: exposure not used in analysis reported	Aldrich et al. (2016)

Table 4.8 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)	Covariates controlled	Comments[b]	Reference
Lung injury based on abnormal spirometry	NA	WTC firefighting USA (New York), WTC firefighters, pre/post (followed up by 6 months post-9/11 with continued follow-up until 2017)	1475 firefighters with and 4264 without lung injury at 6 months post-9/11	BMI, dyslipidaemia, and smoking ↑ risk of WTC-associated lung injury	Age at 9/11, time to follow-up, smoking, race	Longitudinal follow-up and sophisticated statistical modelling are strengths; focus was on modifiers (metabolic syndrome) of the link between WTC and lung injury; no unexposed control group	Kwon et al. (2021)

9/11, WTC disaster on 11 September 2001, New York, USA; Apo-AI, -AII, apolipoprotein-AI, -AII; BALF, bronchoalveolar lavage fluid; BHR, bronchial hyperreactivity; BMI, body mass index; CC16, Club (Clara) cell protein 16; CFA, Country Fire Authority; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CT, computerized tomography; cTnT, cardiac troponins; CVD, cardiovascular disease; DLCO, single breath diffusing capacity of carbon monoxide; EBC, exhaled breath condensate; ECP, eosinophil cationic protein; FEF25–75, forced expiratory flow at 25–75% of FVC; FEV_1, forced expiratory volume (total amount air exhaled) in one second; FSTD, firefighting strategies and tactics drill; FVC, forced vital capacity, total amount air exhaled in 1 breath; GM-CSF, granulocyte/macrophage colony-stimulating factor; GRO, growth-regulated oncogene; HCT, histamine challenge test; HLA, human leukocyte antigen; HR, heart rate; hsCRP, high sensitivity CRP; ICAM-1, intercellular adhesion molecule-1; INF-γ, interferon gamma; IL, interleukin; IP-10, interferon inducible protein-10; LPA, lysophosphatidic acid; MCP-1, monocyte chemoattractant protein; MCT, methacholine challenge test; MDC, macrophage derived chemokine; maxMETs, maximal treadmill exercise testing; MMP, matrix metalloproteinases; MMP-1, interstitial collagenase; MMP-9, interstitial gelatinase; MPO, myeloperoxidase; NA, not applicable; 1-OHP, 1-hydroxypyrene; OR, odds ratio; O_2, oxygen; PAH, polycyclic aromatic hydrocarbon; PM, particulate matter; PMBC, peripheral blood mononuclear cells; PMN, polymorphonuclear neutrophils; PPE, personal protective equipment; ppm, parts per million; PVC, polyvinyl chloride; PTX3, pentraxin; RES, resveratrol; RH, relative humidity; sIL-2R, soluble interleukin-2 receptor; SAA, serum amyloid A; SAR, standardized admission ratio; SCBA, self-contained breathing apparatus; SD, standard deviation; SNP, single nucleotide polymorphisms; SP-A, serum surfactant-associated protein A; SSA, serum amyloid protein; TAC, total antioxidant capacity; TIMP, tissue inhibitors of metalloproteinases; TNFα, tumour necrosis factor alpha; TRF, time-restricted feeding; VCAM-1, vascular cellular adhesion molecule-1; sVCAM, soluble VCAM; VGEF, vascular endothelial growth factor; WTC, World Trade Center; WTC-LI, World Trade Center lung injury; vs, versus; yr, year.

[a] Type of fire may include wildland, wildland emissions, training, municipal, chemical fire, routine firefighting, etc.

[b] Factors to be considered for study quality included the methodology and design, reporting, and exposure assessment quality.

↑, increase; ↓, decrease.

Smith et al. (2019) also observed significant increases in P-selectin and matrix metalloproteinase-9 (MMP-9) in 24 male firefighters after exposure to live-fire structure firefighting drills. [The Working Group noted that this finding of PM-induced MMP-9 generation has been observed in airway epithelial cells where it lasted for 48 hours (Morales-Bárcenas et al., 2015).]

Watt et al. (2016) reported significantly higher baseline IL-6 and neutrophil concentrations in fire instructors, from the United Kingdom, than in healthy controls. After a 7-week no-heat exposure period, levels of IL-6, neutrophils, and lymphocytes were significantly reduced in the fire instructors. [The Working Group noted that this initial elevation in the baseline IL-6 concentration may be because of exposures before testing, and reflective of occupational exposure as a fire instructor.] After the 7-week no-heat exposure period, participants completed a 4-week training course during which there were 15 exposures to live training fires. After the first heat exposure of the training course, IL-6 was again significantly elevated in the fire instructors. Baseline IL-6 concentrations were still significantly elevated at week 4 compared with week 1 before the final heat exposure. Before exposure at week 4, IL-6 concentrations in fire instructors were also significantly higher than those of the control group, although they were still significantly lower than their original baseline measures. After fire exposure at the end of the training week, IL-6 levels were not significantly different from the initial baseline levels before the washout period. [The Working Group noted that although there was only a small sample group (n = 12), and a lack of control over the heat exposure and the specific tasks completed, the pre/post trial study design demonstrated the temporal relationship between the measurements and the firefighters' exposures.]

[The Working Group noted that, for the studies available throughout the present section (Section 4.1.4), repeated (and cumulative) exposures could be regarded as similar to chronic types of exposure. The Working Group also noted that fire instructors, who lead training exercises, are exposed in a similar manner to firefighters; however, they commonly oversee several live-fire exercises in a given day, and these are often repeated over several weeks, year after year (Fent et al., 2019). This suggests that fire instructors' cumulative exposures may be higher and more frequent. The Working Group considered that repeated inflammation may be expected among fire instructors and, over time, could be considered as chronic inflammation.]

Cordeiro et al. (2021) exposed 37 volunteer firefighters, from São Paulo, Brazil, to high temperatures and by-products of combustion in a structure fire simulator that exceeded 600 °C, for 20–30 minutes, and repeated two to three times per day, twice per week, for 4 weeks, as part of a structure training course. This exposure to high temperatures and PM was found to elicit an acute inflammatory process in the airways, with samples of nasal lavage and sputum showing significant acute increases in concentrations of pro-inflammatory and anti-inflammatory cytokines; IL-8; IL-10; IL-2; and the ratio of IL-12p40:IL-12p70. These markers of inflammation had returned to baseline by the end of the training course; however, CC16 concentrations were significantly higher at the end of the 4-week training course than at baseline, indicating possible lung injury. [The Working Group noted that the strengths of this study were the pre/post study design and the longitudinal sampling of nasal lavage and sputum samples to measure airway markers of inflammation.]

In two studies, conducted in Arizona, USA, the impact of smoke exposure during overhaul on markers of inflammation was investigated (Burgess et al., 2001, 2002). Changes in lung function, and in serum CC16 and surfactant-associated protein A (SP-A) were associated with concentrations of specific products of combustion (Burgess et al., 2001). [The Working Group

noted that the study by Burgess et al. (2001) was limited to the evaluation of overhaul exposure and to comparing effects in firefighters wearing air-purifying respirators and no respiratory protection.] In Burgess et al. (2002), there was a significant decline in sputum IL-10 concentration (70%) and mean lung function (forced vital capacity, FVC), after exposure to smoke during an overhaul. Significant increases in serum CC16 and SP-A concentrations were observed; however, these changes were not correlated with IL-10 measures. No significant changes occurred in concentrations of IL-8 and TNFα, despite the fact that IL-8 levels almost doubled. [The Working Group noted that changes in IL-10 concentrations after smoke exposure may result in changes in other inflammation mediators (including IL-8 or TNFα; Burgess et al., 2002) within the lung, which can lead to chronic respiratory effects. Additionally, the significant increases in CC16 and SP-A were indications of increased lung permeability after smoke exposure.]

Gaughan et al. (2014b) reported that increases in high-sensitivity C-reactive protein (CRP) were associated with a decrease in lung function in a cross-sectional study of 401 career firefighters from the midwestern region of the USA. [The Working Group noted that the finding of this study was relevant because CRP has been linked to the development of ischaemic heart disease and stroke, the two primary causes of death in individuals with chronic obstructive pulmonary disease (COPD). These are all chronic inflammatory conditions that have previously been reported in firefighters. However, in the absence of a control group, this finding should be interpreted with some caution.]

[The Working Group noted that all these studies reported significant changes in markers of acute inflammation after structure fire exposure. The strength of some of these papers lay in the study design, which included measures of inflammatory biomarkers collected both pre- and post-fire exposure. The elevations in markers of lung injury suggested tissue damage and chronic inflammation.]

(ii) Wildland fires

Gianniou et al. (2018) completed a thorough clinical assessment and compared markers of inflammation in induced sputum, serum, and bronchoalveolar lavage (BAL) fluid in 60 wildland (forest) firefighters who had completed several consecutive days of firefighting, during the 2008 fires in Greece, and again during the off-season, approximately 3 months after the exposure. The results indicated that eosinophilic and neutrophilic inflammation was evident in the bronchial airways after acute exposure to forest firefighting. Forest firefighting for > 10 hours induced a more intense systemic inflammation than did < 10 hours exposure. Inflammatory cytokine markers were significantly higher after occupational exposure than during the off-season, indicating an acute inflammatory response that did not appear to persist into the off-season (Gianniou et al., 2018). Regarding lung function, forced expiratory flow (FEF) at 25–75% of predicted FVC (FEF25–75), and forced expiratory volume in 1 second (FEV_1)/FVC were significantly reduced post-exposure, with an increased prevalence in respiratory symptoms compared with off-season. However, there was no significant difference in bronchial hyperreactivity off-season and post-exposure. [The Working Group noted that a strength of the study was the thorough clinical assessment of participants. The Working Group noted that these data were indicative of airway and systemic inflammation after a 7-day exposure period; however, the lack of a true baseline measure before deploying to the forest fires was a limitation. It was also hard to control for any additional exposure in the 3-month interim period before the follow-up sample was collected.]

Cherry et al. (2021) conducted a repeated measures study with 160 firefighters after the Fort McMurray fire disaster, a 3-month fire in

Alberta, Canada. Of a panel of 42 inflammatory markers, levels of 25 markers were significantly higher in samples collected in the first 19 days than in samples collected 16–20 weeks later. Clustered within fire service, cumulative exposure, dehydration, and time since last deployed to a fire were all related to late cluster scores of inflammatory markers, as assessed by principal component analysis (PCA). It was concluded that concentrations of inflammatory markers were related to estimates of exposure and decreased with time away from the exposure. [The Working Group noted some limitations with this study: samples were collected from two different locations, at different time-points, and no baseline samples were collected pre-exposure. The nature of the deployments also differed between stations, although estimates of exposure to PM were provided in the appendix to the manuscript.]

Significant increases in IL-8 concentrations pre- to post-shift were reported in three studies conducted in British Columbia, Canada; the Savannah River site, South Carolina, USA; and the Victoria region, Australia; respectively (Swiston et al., 2008; Hejl et al., 2013; Main et al., 2020). The increase in IL-8 was significantly higher in firefighters who spent > 75% of the work shift lighting the fires, as opposed to those who were completing other activities such as "holding" (i.e. maintaining the fire within pre-established boundaries) or "mop-up" (i.e. extinguishing actively smouldering areas) (Hejl et al., 2013). [The Working Group noted that the increase in IL-8 levels observed by Hejl et al. might be because of exposure to the lighter fluid (diesel: gasoline ratio, 3:1) used during the work shift.] Swiston et al. (2008) also showed evidence of inflammatory markers (i.e. granulocytes) in sputum samples collected from forest firefighters after a work shift. Serum concentrations of IL-6, IL-8, and monocyte chemoattractant protein (MCP-1) were also significantly increased after firefighting activity, indicating a systemic inflammatory response after occupational exposure to seasonal forest fires. Estimated exposure to PM was high (peak levels, > 2 mg/m^3), and 65% of the firefighters reported acute respiratory symptoms after the 8-hour shift. A significant increase in plasma IL-8 and IL-6 levels was also observed after a 12-hour shift of wildfire suppression activities associated with the 2009 "Black Saturday" natural disaster in Victoria, Australia. This effect was also accompanied by a significant decrease in IL-10 levels (Main et al., 2020).

Main et al. (2013) reported changes in plasma inflammatory markers across two consecutive days of live-fire suppression tasks (i.e. controlled forest burning) in Australia. It was found that several inflammatory markers changed significantly between pre- and post-shift measurements after a 12-hour shift (i.e. IL-1β, IL-5, IL-7, IL-10, and TNFα). Some inflammatory markers that presented an attenuated response on day 2 were IL-1β, IL-7, IL-4, IL-6, IL-8, and IL-13. [The Working Group noted that although a strength of this study was the repeated measures across two consecutive days, the lack of exposure data represented its limitation. In both instances, these data were indicative of an acute inflammatory response.]

Conversely, Wu et al. (2020b) reported no significant changes pre/post-shift for any of the inflammatory markers, when using EBC to measure cytokines. [The Working Group questioned the sensitivity of the EBC method to measure these biomarkers.]

[The Working Group noted that although most of these studies reported significant changes in markers of inflammation after wildland fire exposure, these effects were primarily acute with limited opportunities to follow up the assessment of chronicity.]

(iii) Employment as a firefighter

Evidence that acute exposure to fire smoke induces an acute neutrophilic airway and long-lasting systemic inflammation in otherwise healthy municipal firefighters was presented by

Greven et al. (2012). Nearly half (44%) of the participants (37 volunteer, 8 career, and 6 as both) reported elevated sputum neutrophil levels (> 60%) that were positively associated with serum IL-8, IL-10, and TNFα concentrations within 24 hours of exposure. A significant increase in serum IL-8 at 24 hours, and at 1 week post-exposure and 3 months post-exposure compared with pre-exposure was observed, as well as a significant increase in serum IL-6 concentrations at 3 months post-exposure. In addition, perceived exposure (i.e. the use of self-contained breathing apparatus, SCBA, and self-reported discomforting exposure to fire smoke) was positively associated with IL-8 concentrations, which were still significantly higher 1 week after exposure compared with baseline. A weak positive correlation was observed between post-exposure levels of neutrophils and particle counts in induced sputum. [The Working Group noted that the strength of this study was the longitudinal follow-up design measuring end-points that could be considered representative of chronic inflammation from both sputum and serum samples. Therefore, the Working Group considered that this study was particularly informative for this key characteristic. Although there was no information on exposure during the 3-month period between samples, airway neutrophilia is a common feature of many chronic inflammatory lung diseases and is associated with disease progression (Jasper et al., 2019).]

Evidence of the long-term effects of occupational exposure on airway and systemic inflammation in firefighters was reported by Gianniou et al. (2016). A thorough clinical assessment was conducted in three groups: career firefighters, trainee firefighters, and healthy controls. The results indicated that inflammatory markers (IL-8, eosinophil cationic protein (ECP), vascular endothelial growth factor (VEGF), and TNFα) in sputum supernatants from career firefighters were significantly higher than in samples from trainees. Serum IL-8 and TNFα concentrations were also significantly higher in the career firefighters than in the trainees. In addition, significantly higher levels of sputum and serum IL-8 and TNFα were reported for the trainees than for the healthy controls. [The Working Group noted that even with relatively short occupational exposure (≤ 1 year), there was a measurable increase in inflammatory markers.] In addition, longer duration of time in service was correlated with higher number of leukocytes in sputum and BAL fluid. From the bronchial biopsy samples provided, there was evidence of mild thickening of the basal membrane and focal increase of mucous production in all career firefighters, with trainees also exhibiting mild thickening of the basal membrane, and small increases in mucus production in almost all samples. The presence of eosinophils was greater in career firefighters than trainees from these tissue samples. Of note, the detection of allergic bronchial sensitization documented by the presence of atopy, and eosinophilia in induced sputum, BAL, and bronchial biopsies are all indicative of chronic inflammation. These results indicated that the effect on bronchial and systemic inflammation was augmented by factors reflective of extended exposure in career firefighters. [The Working Group noted that this study was particularly informative because of the extensive phenotyping and consistency of results (i.e. higher levels of eosinophils in both sputum and BAL), the parallel measurements of biomarkers in sputum and serum, and the use of employment categories used for effect comparisons. Recent evidence suggested that eosinophilia may be a cause, rather than a consequence, of lung cancers in some populations (Wang et al., 2022). However, the potential for self-selection bias was a limitation of the study because only career and trainee firefighters provided biopsies.]

[The Working Group noted that collectively the findings from Greven et al. (2012) and Gianniou et al. (2016) suggest the presence of

long-lasting bronchial and systemic inflammation in career firefighters.]

Watkins et al. (2021) was the only study on occupational exposure as a firefighter to investigate the number of fire heat exposures as the precipitating factor leading to an inflammatory response. Several inflammatory markers were analysed in samples from 110 fire service personnel (53 fire service instructors, and 57 career firefighters). Levels of neutrophils, IL-6, IL-1β, and CRP were significantly higher in fire service instructors than in firefighters. Multiple regression analysis revealed that inflammatory markers could be explained by the number of heat exposures per month. Instructors with > 9 heat exposures per month were 6–12 times as likely to be classified as "at risk" of cardiovascular disease or myocardial infarction and had biomarkers above healthy ranges. [The Working Group noted that this study was particularly informative because it apparently demonstrated a relationship between the inflammatory markers and number of exposures. However, the limitations of the study were self-reported exposures and the cross-sectional design.] Watkins et al. in a previous study (Watkins et al., 2019b) reported that fire service instructors had elevated baseline levels of inflammatory markers (i.e. IL-6 and IL-1β) compared with non-exposed controls. [The Working Group noted the matched healthy control group, and well-controlled study design as strengths of this study; however, the number of self-reported exposures might have been misclassified.]

Andersen et al. (2018a) reported a significant increase in vascular cellular adhesion molecule-1 (VCAM-1) and a decrease in lung function after participation in fire extinction activities. [The Working Group noted that IL-6 and IL-8 were below the levels of detection; however, this did not affect the results for VCAM-1 since it is usually expressed only after endothelial cells are stimulated by cytokines.] Andersen et al. (2018b) observed no changes in VCAM-1 levels or lung function after 3 days of live-fire training exercises, although CRP levels were statistically significantly increased after firefighting training when compared with the control samples collected 2 weeks after the firefighting training [The Working Group noted that these findings were suggestive of acute inflammation.]

A significant increase in interleukin-1 receptor antagonist (IL-1RA) and sputum macrophage count was associated with a slower rate of decline in lung function (Josyula et al., 2007). [The Working Group noted that systemic IL-1RA is natural inhibitor of IL-1β, thus the finding may be indicative of inflammation. However, sputum samples for the assessment of cytokine concentrations were collected at a single time-point only, and exposure history was not reported.]

A series of publications added to the extensive literature on the association between chronic inflammation and occupational exposure as a firefighter employee, although they had some flaws. Four studies reported clinical outcomes apparently associated with chronic inflammation in firefighters. Bergström et al. (1988) published a case report of a firefighter who developed chronic severe asthma that was fatal 25 months after onset. Bodienkova & Ivanskaia (2003) reported significant increases in IL-2, IL-6, IL-1β, and TNFα levels in firefighters with various forms of encephalopathy. Orris et al. (1986) presented case reports of two firefighters who developed chloracne after exposure to a silicon tetrachloride spill (see also Section 4.1.5). Kern et al. (1993) reported on a highly unique cluster of four cases of sarcoidosis (a disease characterized by the growth of collections of inflammatory cells –granulomas – in the body, most commonly in the lungs and lymph nodes) from a cohort study in Rhode Island, USA. [The Working Group noted that these studies, despite not clearly demonstrating mechanistic evidence of chronic inflammation from occupational exposure as a firefighter, presented examples of

diseases associated with chronic inflammation in firefighters.]

Several pre/post trial studies investigated therapeutic treatments to offset inflammation in firefighters, in acknowledgement of the emerging risk of inflammatory markers compromising firefighter health (i.e. precipitating cardiovascular disease and cardiovascular events, respiratory ill health, and acute and chronic lung function impairment) (Barceló-Coblijn et al., 2008; Macedo et al., 2015; Smith et al., 2019; Sotos-Prieto et al., 2019; Diaz-Castro et al., 2020a; McAllister et al., 2020, 2021). [The Working Group noted that these studies did not demonstrate clear mechanistic evidence of chronic inflammation from occupational exposure as a firefighter, rather they focused on the efficacy of these interventions to reduce the acute inflammatory response to firefighting.]

One paper reported a significant interaction effect between cognitive function (attention), inflammatory markers IL-6 and CRP, and alcohol consumption (Yun et al., 2021). [The Working Group noted that the alcohol consumption in this population may be indicative of a negative coping strategy. However, the cross-sectional study design did not allow for the assessment of alcohol consumption as a contributing factor for chronic inflammation.]

(iv) Exposure to heat, or mental and/or physical challenge

Several pre/post trials (n = 10) investigated the inflammatory responses of firefighters to different occupational stressors, such as heat, smoke, humidity, physical exertion or specific firefighting tasks, sleep restriction, and cognitive load, at multiple data time-points. [The Working Group noted that simulation or controlled pre/post trial designs have enabled the research community to specifically investigate the impact of different fireground stressors on inflammatory markers in firefighters.] There was evidence that the individual stressors such as PM (Ferguson et al., 2016), heat stress (Walker et al., 2015; Wolkow et al., 2017; Watkins et al., 2019b), humidity (Wright-Beatty et al., 2014), strenuous physical activity (Webb et al., 2011; Wolkow et al., 2015a), restricted sleep (Wolkow et al., 2015a), decision-making (Huang et al., 2010a), and a combination of different factors or stressors (Smith et al., 2019) induce significant acute inflammatory responses (see Table 4.8).

Walker et al. (2015) reported significant increases in levels of leukocytes and neutrophils pre- to post-exposure to a repeated work protocol task in a heat chamber (100 °C ± 5 °C). Number of cells returned towards baseline within 24 hours of exposure. From the same cohort of municipal firefighters, higher lean body mass was associated with significantly lower values of TNFα at all time-points (Walker et al., 2017). [The Working Group noted that sustained increases in levels of leukocytes and platelets may also increase the risk of cardiac events in firefighters when performing repeat work tasks in the heat, which is particularly relevant during multi-day deployments after natural disasters.]

Wolkow et al. (2015a, b, 2017) examined the impact of repeated days of simulated wildfire suppression tasks on markers of inflammation in volunteer firefighters, with and without the additional stressors of restricted sleep (Wolkow et al., 2015a) and heat exposure (Wolkow et al., 2017). Collectively, these papers indicated that diurnal levels of IL-6 were above normal ranges in these volunteer firefighters, and IL-6 significantly increased across the 3-day study period (Wolkow et al., 2015a). Increases in morning IL-6 levels were associated with a significant increase in evening cortisol in sleep-restricted firefighters, and a daily increase in cortisol levels across the 3-day study period (Wolkow et al., 2015b) (see Section 4.1.6). IL-8 levels were also significantly higher in the groups of firefighters who had 8 hours of sleep compared with those who had 4 hours (Wolkow et al., 2015a), whereas IL-4 was significantly higher under hot

conditions (ambient temperature controlled at 33 °C) (Wolkow et al., 2017).

[The Working Group noted that the occupational exposure studies, presented earlier, focused on the inflammatory consequences of smoke, PM exposure, and the implications or evidence for the development of associated respiratory complaints. In contrast, the simulation training and pre/post trial studies focused mainly on elucidating the inflammatory mechanisms underpinning the risk of cardiovascular disease or sudden cardiac events. The health effects of repeated occupational exposure to heat are yet to be understood. The work on fire service instructors suggested that these individuals develop maladaptation to repeated fire exposures, showing elevated resting cytokine levels and an increased prevalence of symptoms of ill health (Watkins et al., 2019b).] Also, Huang et al. (2010a) observed changes in pro-inflammatory cytokine IL-2 levels, but not IL-6 levels, in firefighters exposed to a decision-making challenge (firefighting strategies and tactics drill) while participating in moderate intensity exercise (see also in Section 4.1.5).

(v) Catastrophic events

Firefighters from the WTC cohort, with elevated levels of CRP within 6 months of the event, had a significantly increased risk of developing decreased lung function (FEV_1) as assessed by subsequent pulmonary testing (Weiden et al., 2013). Induced sputum from firefighters who were highly exposed to the WTC dust revealed significantly higher cell counts (i.e. macrophages, neutrophils, lymphocytes, and eosinophils) 10 months after the event than those for a control group of health-care workers from Tel Aviv who were not exposed to WTC dust (Fireman et al., 2004). There was no significant difference in cell counts between the two firefighter cohorts, although the cell counts for firefighters in both cohorts were significantly higher than those for the respective health-care workers. [The Working Group noted that even without the exposure to WTC dust, all firefighters presented with significantly higher cell counts than did the control group. This was in spite of significant differences in the particle analysis and percentage of samples with different particle sizes between induced sputum from both populations.]

Several studies reported on the WTC-exposed cohort. Clinical investigations and nested case–cohort studies focused on the relationship between dust and PM exposure from the WTC disaster and subsequent acute and chronic inflammation-derived respiratory effects experienced in the WTC-exposed population (e.g. Kwon et al., 2013; Nolan et al., 2014; Zeig-Owens et al., 2018), from bronchial hyperreactivity (Aldrich et al., 2016), chronic rhinosinusitis and sinus polyps (Cho et al., 2014), and COPD (Singh et al., 2018) to chronic inflammatory polyarthritis (Loupasakis et al., 2015) and sarcoidosis (Loupasakis et al., 2015; Hena et al., 2018; Cleven et al., 2019). [As mentioned in Section 4.1, the findings from the WTC-exposed cohort may not be generalizable to other firefighting populations because of the massive acute exposure to WTC dust, which differed from dust from other live fires in terms of its PM composition. The Working Group noted that possible unmeasured events before or after WTC exposure could have also affected the results.]

Levels of IL-8 and TNFα and polymorphonuclear neutrophils (PMN) count were all significant predictors of sinus disease severity in firefighters exposed to WTC dust (Cho et al., 2014). Singh et al. (2018) further identified several inflammatory markers that represented risk factors for the subsequent development of irritant-associated asthma/COPD overlap (i.e. the firefighter developed both asthma and COPD). Specifically, elevated serum eosinophil and IL-4 levels were associated with subsequent asthma/COPD overlap. Greater serum IL-21 concentration was also associated with the development of isolated

asthma and isolated COPD in WTC-exposed firefighters (Singh et al., 2018).

Nolan et al. (2012) indicated that mediators of metabolic syndrome, inflammation, and vascular injury in early serum samples were predictive of lung function in the WTC-exposed cohort. Tsukiji et al. (2014) drew on this same population to investigate risk of developing WTC lung injury. It was found that increased levels of lysophosphatidic acid (LPA) and apolipoprotein-AI (ApoA1) in serum were significant predictors of WTC lung injury-associated loss of FEV_1 when sampled within 6 months after the WTC event and when adjusting for several factors, including PMN count (Tsukiji et al., 2014).

Lam et al. (2020) compared data for nine analytes in serum collected within 200 days of exposure from 15 WTC-exposed first responders who, up to 16 years later, had a defined lung injury; the authors also included 15 controls. The firefighters were non-smokers who had normal lung function before the WTC event and who, during the 16-year follow-up, were identified as having a lung injury if their percentage of predicted forced expiratory volume ($FEV_{1,\%Predicted}$) was less than the lower limit of normal, as defined by the National Health and Nutrition Examination Survey III. Control firefighters had an $FEV_{1,\%Predicted}$ that was greater than or equal to the lower limit of normal. There was a significant difference in the concentration of one analyte only, chemokine MCP-1, which was found at higher concentrations among the exposed group (Table 4.8).

Using these data, Lam et al. (2020) performed a correlation matrix and found strong correlations between LPA and soluble receptor for advanced glycation end-products (RAGE); strong correlations among various cytokines/chemokines, including interleukins IL-1α, IL-8, and IL-10, macrophage inflammatory protein (MIP-1α), granulocyte/macrophage colony-stimulating factor (GM-CSF), and TNFα; and negative correlations between many of these cytokines/chemokines and several sphingolipids and omega fatty acids (Table 4.8). [The Working Group noted that LPA and RAGE have key roles in the development of lung injury related to WTC exposure. In addition, the inflammatory response could be partly a result of dyslipidaemia-driven inflammation.]

In the same study, Lam et al. (2020) exposed the human THP-1-derived macrophages to WTC-PM_{53} (≤ 53 μm) at 100 μg/mL for an acute exposure of 24 hours and found increased levels of GM-CSF, IL-8, IL-10, and MCP-1. These results showed that WTC-PM_{53} induced inflammation biomarkers in human cells in vitro. Co-exposure to WTC-PM_{53} plus LPA resulted in a synergistic decrease in expression of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB), protein kinase B (*p*-Akt), and STAT3/5 signalling. In addition, in vitro acute exposure of the cell line RAW264.7 mouse-derived macrophages for 24 hours to WTC-PM_{53} increased levels of various cytokines, such as IL-1α, TNFα, NF-κB, and IL-10 (Lam et al., 2020). In vitro acute exposure of these cells to WTC-PM_{53} plus LPA resulted in a synergistic decrease in expression of NF-κB, *p*-Akt, and STAT3,5b). [This in vitro study was not designed to address the issue of chronic inflammation and does not provide useful information relative to chronic inflammation.]

In addition, three studies reported on cases of sarcoidosis among firefighters exposed to WTC dust (Loupasakis et al., 2015; Hena et al., 2018; Cleven et al., 2019). Hena et al. (2018) described the clinical course of sarcoidosis in firefighters followed up 8 years after diagnosis. Loupasakis et al. (2015) reported on 11 case examples of sarcoidosis with polyarticular arthritis. Diagnoses of sarcoidosis were based on clinical, radiographic, and pathological criteria. Nine of the 60 firefighters who developed sarcoidosis after 11 September 2001 (9/11) presented with polyarticular arthritis, there were a further two cases diagnosed before 9/11 in firefighters who

developed sarcoid arthritis after WTC exposure, and all had biopsy-proven pulmonary sarcoidosis (Loupasakis et al., 2015). [The Working Group noted that from the emergent data on the 11 case examples presented with biopsy-proven sarcoid arthritis, it was concluded that chronic inflammatory polyarthritis appears to be an important manifestation of sarcoidosis in firefighters with WTC exposure and sarcoidosis.]

Genetic susceptibility is also an important molecular factor to consider in the associations between exposures and ultimate risk of cancer. Cleven et al. (2019) examined genetic susceptibility to sarcoidosis among cases that developed because of WTC-related exposures. All cases (n = 55) and non-sarcoidosis controls (n = 100) were selected who were followed up for 14 years after the WTC disaster. A custom panel was used to fully sequence 51 genes involved in immune response, inflammation, granuloma formation, and general risk of sarcoidosis. Among 3619 common variants detected among all participants, 17 were significantly more common among sarcoidosis cases and 764 specifically among extrathoracic sarcoidosis cases. [The Working Group noted that this study demonstrated the potential for gene–environment interactions in occupational disease. This may in part explain the highly unique cluster of four sarcoidosis cases from the Rhode Island cohort observed in Kern et al. (1993).]

[The following studies reviewed by the Working Group were considered less informative for the key characteristic "induces chronic inflammation" since the protocol did not allow specific conclusions to be made regarding changes in biomarkers of chronic inflammation and exposure to structure fires or employment as a firefighter: Kudaeva & Budarina (2005, 2007); Barceló-Coblijn et al. (2008); Wolkow et al. (2016a, b); Adetona et al. (2017); Sotos-Prieto et al. (2019); McAllister et al., 2020). For this reason, they were not reviewed in Section 4.1.4.]

(b) Alteration in lung function processes and bronchial hyperreactivity

Among the available studies providing information on chronic inflammation, several reported altered lung function after occupational exposure to smoke or PM. This was often used as a proxy for lung injury, which may be indicative of an inflammatory state. From the papers reviewed, it is suggested that these exposures lead to a significant decline in lung function associated with alterations in inflammatory markers (Burgess et al., 2004; Gaughan et al., 2014b; Andersen et al., 2018b; Gianniou et al., 2018; Zeig-Owens et al., 2018), pneumoproteins (Burgess et al., 2001, 2002; Greven et al., 2011a), or respiratory symptoms (Greven et al., 2011b, c).

[The Working Group noted that impairment of lung function could be partly explained by changes observed, through clinical investigations, in the lower airway tract, although it is not clear to what extent the observed inflammatory response and pathological changes represent permanent damage or are part of a natural temporary defence mechanism. The transition to a permanent condition may depend on the duration or extent of the exposure, as reported in the studies from Gianniou et al. (2016) and Watkins et al. (2021), and the subsequent damage. In addition, it has been suggested that the degree of lung function decline can be also explained by variations in genes involved in inflammatory responses, which would account for observed interindividual variability (Burgess et al., 2004; Josyula et al., 2007; Yucesoy et al., 2008).]

There were several papers that reported changes in lung function in the absence of specific markers of chronic inflammation. Greven et al. (2011c) reported an association between respiratory symptoms and fire exposure or smoke inhalation. There was a significant relationship between bronchial hyperreactivity and the number of fires fought in the last 12 months (Greven et al., 2011b). CC16 protein

was inversely associated with the number of fires fought in the last 12 months, and this association grew stronger when adjusting for lung function (Greven et al., 2011a). [The Working Group noted that a decrease in CC16 levels is often observed in individuals with asthma, and although there was a trend for an association with firefighters diagnosed with asthma in the current study, it was not significant.] Four studies assessed lung function (by spirometry) without additional measures (Loke et al., 1980; Burgess et al., 1999; Almeida et al., 2007; Kwon et al., 2021).

In a non-smoking group of 22 firefighters, 4 had evidence of obstruction of the airways. This disease of the small airways was only present in firefighters with > 25 years of experience. Irreversible lung injury was present in one firefighter who had been trapped in a basement fire (Loke et al., 1980). [The Working Group noted that, although the self-reported exposure is a limitation of the studies, these results are indicative of persisting respiratory symptoms and lung injury or damage after smoke inhalation and are therefore suggestive of chronic inflammation.]

The Working Group reviewed studies of firefighters that included outcomes relevant to allergic airway sensitization (i.e. presence of atopy and bronchial hyperreactivity) and/or increased respiratory symptoms (wheezing, cough, chest tightness, sneezing, and expectoration), since these outcomes can be relevant in the development of cancers of the respiratory tract (see Section 2.1). Occupational exposures as a firefighter included various airborne chemical agents, some of which are carcinogens or potential carcinogens (e.g. PM, VOCs, sVOCs, PAHs, asbestos, PFAS, etc.), with inhalation being the predominant route of exposure (see Section 4.1 and Sections 1.4.1, 1.4.4, and 1.5.1). Seven studies in humans were identified that assessed bronchial hyperreactivity, atopy, allergic rhinitis, and/or respiratory symptoms (Chia et al., 1990; Herbert et al., 2006; Greven et al., 2011b, c; Aldrich et al., 2016; Gianniou et al., 2016, 2018). Many of these studies also measured immune–inflammatory markers; these markers are also discussed above.

Several studies assessing bronchial hyperreactivity are briefly discussed here, as the Working Group deemed this an important outcome relevant to chronic airway inflammation. Bronchial hyperreactivity was assessed in relation to employment as a firefighter (Greven et al., 2011b, c; Gianniou et al., 2016), wildland firefighting (Gianniou et al., 2018), and among firefighters and other first responders to the WTC event (Aldrich et al., 2016). Comprehensive tests for bronchial inflammation and hyperreactivity among groups of professional and trainee municipal firefighters and non-firefighter controls were compared (Gianniou et al., 2016). Professional firefighters had a higher prevalence of atopy, allergic rhinitis, and bronchial hyperreactivity than did trainees and healthy controls. Among a large sample of firefighters from brigades throughout Denmark, the number of fires fought in the past 12 months was positively associated with bronchial hyperreactivity. This association was stronger among individuals with atopy (Greven et al., 2011b). Greven et al. (2011c) noted a positive association between an inhalation incident and respiratory symptoms related to bronchial hyperreactivity among 1330 firefighters in Denmark. [The Working Group noted that both exposure and symptoms were self-reported; bronchial hyperreactivity was not measured directly.] Bronchial hyperreactivity was assessed in wildland firefighters within 1 week of exposure and compared with samples collected 3 months later in the off-season (Gianniou et al., 2018); no differences in bronchial hyperreactivity or provocation over time were noted.

Aldrich et al. (2016) provided long-term follow-up of WTC responders. New York firefighters with no documented asthma and normal spirometry before the event who also participated in subsequent follow-ups (2 years and > 10 years after the event) were included (n = 173). Bronchial hyperactivity was seen in

16% of firefighters at 2 years after the WTC event and in 25% of firefighters at the second follow-up. Participants with bronchial hyperreactivity at the first follow-up had more respiratory symptoms, abnormal FEV_1, and provocability. [The Working Group noted that although the authors suggested that a selection bias may have occurred and the protocol to measure bronchial hyperreactivity changed between the two follow-ups, the results indicated potential long-lasting changes to airway hyperreactivity among WTC-exposed firefighters.]

[The Working Group noted that collectively these studies suggest that bronchial hyperreactivity is an outcome associated with firefighting. These data were considered alongside findings described previously, since this outcome is relevant to chronic inflammation of the airways.]

4.1.5 *Is immunosuppressive*

(a) Exposed humans

See Table 4.9.

Lymphocyte counts, lymphocyte subsets, and immunoglobulin levels were the end-points considered relevant to the key characteristic "is immunosuppressive" and reported in this section. An increase in lymphocyte count is indicative of leukocytosis (noted in Section 4.1.4), whereas a decrease suggests immunosuppression. Reduced immunoglobulin levels may also indicate immunosuppression, increasing the risk of infection; whereas an increase in immunoglobulin levels suggests upregulation of humoral immunity, current infection, or increased allergy sensitivity.

Twenty studies available to the Working Group evaluated mechanistic end-points relevant to immunosuppression after occupational exposure as a firefighter. Studies assessed a variety of exposure types, including structure fires (mainly training; five studies), wildland (forest) fires (one study), employment as a firefighter (four studies), exposures with a heat, mental, or physical challenge (six studies), and catastrophic events (four studies). Two additional studies investigated the prevalence of infection with the SARS-CoV-2 virus (the cause of COVID-19 disease) among firefighters.

(i) Structure fires

A controlled training fire exercise resulted in increased lymphocyte counts immediately after exposure followed by reduction 90 minutes after exposure. T-lymphocyte proliferation also increased after exposure, although this correlated with increased lymphocyte numbers (Smith et al., 2005). [The Working Group proposed that it was probably altered lymphocyte numbers that caused the proliferation response rather than cell responsiveness.] Watkins et al. (2019a) also reported increased lymphocyte counts immediately after training fire scenarios conducted by instructors, with a positive correlation between change in core temperature and post-exposure lymphocyte values. [The Working Group noted that Smith et al. (2005) replicated firefighting tasks and rest periods across participants, whereas Watkins et al. (2019a) did not control for tasks because collection was performed during training courses. Neither study gave results suggestive of immunosuppression; both studies used small sample sizes and did not include flow cytometric analysis of cell subsets.]

Two studies (Smith et al., 2004; Watt et al., 2016) provided a chronic assessment of structure-fire training exposures. Four days of training fire exposures resulted in increased lymphocyte counts on days 3 and 4 (Smith et al., 2004). Blood samples from instructors revealed reduced lymphocyte counts after a 7-week break from fire exposures, but no further changes were detected immediately after fire exposure or 4 weeks after instructing a course (Watt et al., 2016). Immunoglobulin G (IgG) levels immediately after exposure decreased after a 4-week instructing course compared with pre-course levels. However, comparison of blood samples from instructors with those from an age-matched

Table 4.9 End-points relevant to immunosuppression in exposed firefighters

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Structure fires							
Lymphocyte count, lymphocyte proliferative response	Blood	Structural [municipal] firefighting (training) USA, male firefighters, pre/post trial	11, 0	↑ No. of lymphocytes post ($P < 0.001$) ↓ Lymphocytes after 90 min ($P < 0.05$) ↑ Proliferation post ($P < 0.007$)	Diet, firefighting tasks, PPE, rest periods	Small sample size (3 trials consecutively completed, average time to completion 5 min 29 s to 6 min 17 s, 10 min rest between trials 2 and 3) Exposure assessment: appropriate in terms of assessing the effect of firefighting; no specific firefighting hazard assessed	Smith et al. (2005)
Lymphocyte count	Blood	Structural [municipal] firefighting (training) United Kingdom, fire service instructors (14 men, 2 women), pre/post trial comparing training days	16, 0	↑ Lymphocyte count ($P < 0.01$) No changes in lymphocyte count between exposure combinations Positive correlation between change in core temperature and post-exposure lymphocytes ($P = 0.002$)	Menstrual cycle phase for female participants	No non-exposed control group; small sample size; roles and duration of exposure varied between participants (day of exposures, 3-day options, 1 – demo and attack, 2 – multi compartment × 2, 3 – demo, attack and multi compartment) Exposure assessment: exposure to different fire exercises appropriately tested as exposure for the effects assessments that were done in the experiment	Watkins et al. (2019a)
Lymphocyte count	Blood	Structural [municipal] firefighting (training) USA, male firefighters, pre/post-exposure across 4 days, comparison made across exposure and day 1 to days 2, 3 and 4)	16, 0	↑ Lymphocytes after exposure ($P < 0.001$) ↑ Lymphocytes on days 3 and 4 ($P = 0.046$)	Medically cleared for duty	Limited detail on exposure tasks and durations; no non-exposed control group to compare daily fluctuations Exposure assessment: specific firefighting exposure was not evaluated but effect of involvement in firefighting appropriately tested with the study design; possibly prior exposure during the earlier days of training might have confounded results but not enough information to determine if this occurred	Smith et al. (2004)

Table 4.9 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Lymphocyte count, Ig concentrations	Blood	Structural [municipal] firefighting (training) United Kingdom, male firefighters (instructors) and non-exposed controls (university lecturers), pre/post trial	6, 6	↓ Lymphocytes pre to post break ($P < 0.05$) IgG, no difference between instructor and control samples at any time-point	Time since recent exposure, no additional operational exposures; control group no exposure to > 25 °C in previous 4 months	Variation in exposure duration and roles conducted; small sample size Exposure assessment: inadequate since potential simultaneous exposure to smoke was not considered; the quantitative heat exposure measure that was collected was not used in exposure–response analysis	Watt et al. (2016)
Ig concentrations	Blood	Structural [municipal] firefighting (plastic) Sweden, case report (1 male firefighter	1, 0	No change in immunoglobulin		Single time-point post exposure assessed: study focused on development of severe asthma, which led to death Exposure assessment: qualitative description of the exposure due to burning plastic; PPE was not used	Bergström et al. (1988)
Wildland fires							
Lymphocyte proportion	Sputum, BALF	Wildland Greece, firefighters, repeated measures design	60, 0	No changes in lymphocyte proportion several days post-exposure vs 3 months off-season		Visit 1 24–48 h after fire exposure; unclear if PPE was worn; 87% current smokers with history of 9 ± 5 packs/year; participant's sex not detailed; samples stained and manually counted – presented as percentage of non-squamous cells Exposure assessment: time away from firefighting adequate for effects that were tested; potential exposure misclassification for length of firefighting	Gianniou et al. (2018)

Table 4.9 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Employment as a firefighter							
Lymphocyte count	Blood	Employment as a firefighter Canada, male firefighters (≤ 10 yr experience vs ≥ 20 yr experience) and non-exposed controls, cross-sectional	30 (15, 15), 15	Firefighters vs control No changes in Th1 and Th2 ↑ Th17; ↑ Th22; ↑ Tregs ($P < 0.001$) No changes in subsets between firefighter experience groups No correlation between Th17 and Treg in high experience group; correlation was present in lower experience group ($P = 0.0013$)	Non-smokers only	Controls age-matched to firefighters; no information regarding timing of sample to previous exposure Exposure assessment: cross-sectional design with qualitative measures of exposure and potential for confounding by non-firefighting related exposures	Ricaud et al. (2021)
Lymphocyte counts, IgG concentrations	Blood	Employment as a firefighter United Kingdom, firefighters (55 men, 2 women) vs fire service instructors (47 men, 6 women), cross-sectional	57 firefighters, 53 instructors	No changes in lymphocyte counts ↑ IgG in instructors ($P < 0.001$) Regression analysis revealed no association between IgG and age, time in service or weekly exposure number Positive association between IgG and monthly exposure number ($P < 0.05$)	Exercise and fire exposure avoided 12 h before sample collection	Fire exposures and health complaints self-reported; groups matched on age, body mass, and time in service Exposure assessment: self-reported frequency prone to misclassification	Watkins et al. (2021)

Table 4.9 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Lymphocyte proportion, IgE concentration	Blood, BALF	Employment as a firefighter Sweden, male firefighters (≥ 3 yr of experience) and healthy control, cross-sectional	13, 112	↑ Proportion of lymphocytes ($P < 0.05$) No changes in IgE		Unbalanced sample sizes; non-smoking control, but 5 ex-smokers in firefighter group; unclear regarding control occupation and heat/smoke exposure; 9/13 firefighters had performed firefighting in the last 3 months, of these, 4/9 had used PPE; exposures were self-reported; samples stained and counted – presented as percentage of non-squamous cells Exposure assessment: heterogeneous group, some without recent exposures; self-reported number of fires fought may be misclassified	Bergström et al. (1997)
Lymphocyte proportion	Sputum, BALF	Employment as a firefighter Greece, male firefighters, (professional [career] vs part-time 1 yr trainees vs control), cross-sectional	63, 29, 18	No changes in lymphocytes between groups Positive correlation between years of experience and lymphocytes ($P = 0.016$)	Use of respiratory protection reported to be similar between firefighter groups	No information regarding exposure types, frequency, or time since last exposure; smokers included; years of service for professionals [career] was short (9 ± 1 yr); samples stained and counted – presented as percentage of non-squamous cells Exposure assessment: employment categories used for effects comparisons likely adequate; potential confounding of career length with age	Gianniou et al. (2016)

Table 4.9 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Exposure to heat, mental, or physical challenges							
Lymphocyte count	Blood	Mental and physical challenge USA, male firefighters, pre/post trial (laboratory cycling exercise)	9, 9 (Huang et al., 2010a) 10, 10 (Huang et al., 2010b)	No change CD8+ ↑ After exercise CD56+ ($P < 0.001$) ↑ After exercise CD56+ and CD3− NK cells ($P < 0.001$) ↓ After exercise CD3+ T-cells, CD3+ and CD4+ helper T-cells, CD4:CD8 ratio, CD19+ B-cells, and total lymphocytes ($P < 0.001$)	Exercise intensity and duration, cardiovascular disease, smoking status, no fire exposure in previous 72 h	Exercise modality (cycling) not similar to firefighting; small sample size Exposure assessment: engagement in experimental drill exercise appropriately tested as exposure for the effects assessments that were done in the experiment	Huang et al. (2010a, b)
IgG, IgA, IgM concentrations	Blood	Physical challenge Portugal, male firefighter recruits, repeated measures design	24 (12 with and 12 without supplement), 0	No change in IgG, IgA, IgM	Diet, training activities, injury/illnesses	No non-training control group; sample were recruits, baseline levels may not be representative of firefighters; statistical follow-up tests unclear Exposure assessment: engagement in experimental fitness test appropriately tested as exposure for the effects assessments that were done in the experiment in relation to supplement intervention	Santos et al. (2020)
Lymphocyte count, IgG concentrations	Blood	Heat United Kingdom, fire service instructors (9 men, 2 women) vs controls (university lecturers), pre/post trial	11, 11	↑ Lymphocytes in both groups post-exposure ($P < 0.05$) ↑ IgG at rest in instructors vs control ($P = 0.001$)	Control group no exposure to > 25 °C in previous month	Control group matched on age, sex, body fat percentage; small sample size; same response noted same trial conducted 2 months later Exposure assessment: number of self-reported fires may be misclassified; heat exposure was under controlled conditions	Watkins et al. (2019b)

Table 4.9 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Lymphocyte count	Blood	Heat Australia, male firefighters, pre/post trial (pre vs post, 1 h post, 24 h post)	42, 0	↑ Lymphocytes 1 h post-exposure ($P < 0.01$) All markers returned to baseline in 24 h	Exposure temperature, physical tasks and durations, rest periods	Well-controlled design with repeated time-points Exposure assessment: exposure to heat appropriately tested as exposure for the effects assessments that were done in the experiment	Walker et al. (2015, 2017)

BALF, bronchoalveolar lavage fluid; Ig, immunoglobulin; NK, natural killer; PPE, personal protective equipment; vs, versus; yr, year.

[a] ↑, increase in biomarkers, ↓, decrease in biomarkers

[b] Factors to be considered for study quality included the methodology and design, reporting, and exposure assessment quality.

control group revealed no differences in lymphocyte count or IgG levels at any time-point (Watt et al., 2016). [The Working Group noted that the training fire exposures varied in duration and task, and only a small sample size (six instructors) was studied.]

In a case study of an acute exposure to a structure fire (see also in Section 4.1.4) experienced by a firefighter without breathing protection, no change in immunoglobulin levels was reported, although the firefighter developed severe chronic asthma, which ultimately resulted in the incident being fatal (Bergström et al., 1988). [The Working Group noted that because of the case-study nature and lack of breathing protection, it was unlikely that this study provided an accurate representation of typical fire-exposure responses. No samples were available for comparison with pre-exposure levels.]

(ii) Wildland fire

One study assessed the consequences of wildland (forest) fire exposure, revealing no difference in lymphocyte counts in samples collected after several continuous days of firefighting compared with samples collected 3 months into the off-season (Gianniou et al., 2018). [The Working Group noted that limited time-points were assessed, and there was no baseline measurement or cell subset analysis.]

(iii) Employment as a firefighter

Four studies made use of cross-sectional designs to compare firefighters with non-exposed controls. Analysis of immune cell subsets from operational firefighters with varying experience levels (Ricaud et al., 2021) revealed no difference between firefighters and controls in CD4+ T-helper Th1 and Th2 cells. Instead, CD4+ Th22, Th17, and T-regulatory (Treg) cells were significantly increased in firefighters. There was no difference in any subset when firefighters with ≤ 10 years of experience were compared with those with ≥ 20 years of experience. However, correlation between Th17 and Tregs was not present in the group of more experienced firefighters. [The Working Group noted that this absence of correlation may indicate an imbalance in immune homeostasis. Furthermore, it has been reported that in some instances of cancer, tumour cells promote the expansion of Tregs, which leads to a decrease in the anti-tumour immune response (Nishikawa & Sakaguchi, 2014). The Working Group noted that firefighters and controls were age-matched, and all participants were non-smokers.] Comparison between firefighter subpopulations (firefighters versus instructors) demonstrated no difference in lymphocyte counts between groups, but IgG levels were increased in instructors (Watkins et al., 2021). The authors reported that there was no association between IgG and years of experience, as assessed by multiple regression analysis; however, the number of fire exposures per month was associated with IgG. The study also noted increased symptoms of ill health among instructors, including severe fatigue, coughs, and colds; instructors exhibiting values above the reference ranges for IgG were 6.45 times as likely to experience ill health symptoms as those with values below the reference ranges. [The Working Group noted that the increased IgG levels may be representative of increased humoral immunity but highlighted that additional biomarker analysis is needed to investigate the balance between humoral and cellular immunity. In addition, the Working Group noted that the sample size was large, but only a single time-point was measured, and exposures and health were self-reported. The Working Group also noted that respiratory symptoms (such as coughing, wheezing, phlegm, and reduced lung function) have been reported in numerous studies, although study design and measurements have not established a clear link with infection and immunosuppression, instead implicating inflammation as the key pathway (see Section 4.1.4).]

Serum immunoglobulin E (IgE) levels did not differ between male firefighters and healthy controls, but an increase in the proportion of lymphocytes in BAL in firefighters was detected, although values remained within reference ranges (Bergström et al., 1997). Gianniou et al. (2016) reported no difference in lymphocyte counts between active firefighters, trainees of 1 year, and controls, although correlation analysis did reveal an association between time in service and lymphocyte count. [The Working Group noted that detail on exposures was limited and that increase in lymphocyte count could be indicative of inflammation (see Section 4.1.4), not immunosuppression.]

A recent assessment of the prevalence of coronavirus SARS-CoV-2 infection in military firefighters reported that 14–46% of test responses were positive based on immunoglobulin M (IgM) and IgM antibody lateral flow tests or real-time polymerase chain reaction tests (Borges et al., 2021). [The Working Group commented that this prevalence highlighted the importance of immunization for workers who engage with the general population, work within restrictive spaces, and often complete tasks involving physical contact. However, no statistical comparison with a non-fire exposed control group was provided, and there was no information on the fire exposure history of the firefighters included in the study.] Further investigation of SARS-CoV-2 in emergency first responders by Montague et al. (2022) identified no difference in infection prevalence between firefighters and other similar occupations (such as the police and medical staff).

(iv) Exposure to heat, or mental and/or physical challenge

Two studies conducted crossover-controlled laboratory trials (Huang et al., 2010a, b) involving active firefighters who performed a 37-minute cycling exercise with and without a firefighter strategy and tactics drill. Mental challenge did not exacerbate any immune marker responses: exercise both with and without mental tasks resulted in no change in CD3+ and CD8+ cytotoxic T-cells, increased CD56+ (Huang et al., 2010b) and CD3− natural killer (NK) cells immediately after stress, and decreased CD3+ T-cells, CD3+ CD4+ T-helper cells, the CD4:CD8 ratio, CD19+ B-cells, and total lymphocytes (Huang et al., 2010a). All levels recovered to baseline 1 hour after exercise (Huang et al., 2010a). [The Working Group noted that these responses may indicate an increase in the innate immune response but suppression of adaptive immunity in relation to exercise, although the trial did not simulate firefighter tasks in terms of exercise modality, temperature, clothing encapsulation, or smoke exposure. Generalizability to fire scenarios was therefore limited. The Working Group also noted that both studies had small sample sizes, and there was uncertainty regarding the crossover of participants between studies.]

One randomized control trial study assessed recruit firefighters before and after a 5-week training programme. No differences in IgG, IgA, or IgM were detected, and values were within normal ranges (Santos et al., 2020). [The Working Group noted that the training programme did not include any fire suppression activities or a non-training control group. The lack of differences noted in this study did not therefore necessarily represent the consequences of fire-exposure training courses.]

Firefighters' occupational exposure promotes physical exertion and heat stress, which contributes to increased body and skin temperatures (see Section 1.5.1(f)). Watkins et al. (2019b) reported increased lymphocyte counts in fire service instructors and in a control group of university staff after exposure to heat (50 ± 1.0 °C) while wearing protective clothing and exercising for 40 minutes (Watkins et al., 2019b). Lymphocyte counts were not different between groups; however, instructors exhibited elevated IgG levels compared with controls before exercise (Watkins et al., 2019b). [The Working Group noted that the

lack of difference in acute responses between the instructors and the control group may suggest that the magnitude of the lymphocyte response is not altered by a history of repeated exposures. However, the study did not control for other exposures in the control group, besides heat exposure in the previous month.] Assessment of responses to simulated search tasks in a heat chamber (~100 °C) also noted leukocytosis, which included elevated lymphocyte counts from the end of the exposure to 1 hour after exposure (Walker et al., 2015, 2017). Subsequent measurement at 24 hours after exposure revealed that lymphocyte counts had returned to resting levels (Walker et al., 2015, 2017). [The Working Group noted that the studies by Walker et al. used a large sample (n = 42), and by using the simulated scenario were able to control for numerous confounding factors, such as environmental temperature, task type and duration, and rest periods. The inclusion of additional measurement points beyond immediate cessation of exercise provided detail on the time course of responses. The lack of cell subset analysis in these three studies limited the conclusions that could be drawn regarding immunosuppression. The responses reported in these studies were the consequence of physiological strain and heat, not smoke exposure.]

(v) Catastrophic events

Firefighters may experience exposure to chemicals and physical factors during building collapse and other catastrophic events; detailed exposures are presented in Section 1.5.1(g) (Table 1.5.2). Four cross-sectional investigations focused on exposure to specific incidents (Bodienkova & Ivanskaia, 2003; Fireman et al., 2004; Kudaeva & Budarina, 2005, 2007). [The Working Group reviewed the studies by Kudaeva & Budarina, (2005, 2007) but considered them not informative since they did not provide detail regarding the toxic substance exposure and included limited information regarding sample timing and group sizes.] Bodienkova & Ivanskaia (2003) conducted a cross-sectional analysis 7 years after exposure at the 1992 "Irkutskcable" factory fire in Shelekhov, Russian Federation [the Working Group noted that the study did not provide details about the event]. Reduced lymphocyte count, including decreased CD3+ and CD4+ T-helper cells, CD8+ T-cytotoxic cells, and increased IgA in firefighters with encephalopathy compared with non-exposed controls were reported. [The Working Group highlighted that limited detail was available regarding the interim 7-year period, and only exposed firefighters diagnosed with encephalopathy were included in the study (other exposed firefighters were not considered).] Fireman et al. (2004) identified increased lymphocytes in firefighters who attended the WTC event compared with control health-care workers in Israel; however, this elevation was also noted in Israeli firefighters. [The Working Group noted that only a single sample was analysed 10 months after the WTC event, with no details on exposure in the interim period.]

[The Working Group concluded that the complexities of immune regulation are time dependent, and limited subset assessment was available to develop understanding of the balance of upregulation and suppression between innate, humoral, and cellular immunity. The overall evidence did not rule out an association between firefighting and immunosuppression. From the limited studies available, there was some indication that firefighting may be immunomodulatory (as noted in the review of Ricaud et al. (2021) and Watkins et al. (2021)). However, because of a paucity of evidence, the available literature was not sufficient to indicate an immunosuppressive response to firefighting.]

(b) Human cells in vitro

No data were available to the Working Group.

(c) Experimental systems

(i) Non-human mammals in vivo

One experimental transcriptomic model study in mice suggested an immunomodulatory impact of firefighting overhaul exposures when respiratory protection was not used (Gainey et al., 2018) (see Section 4.1.6). In vivo exposure of mice to either flaming or soldering emissions from peat, oak, or eucalyptus suppressed cytokine levels in allergic or non-allergic animals (Hargrove et al., 2019). [The Working Group noted that this was most likely because of smoke-induced suppression of allergic inflammatory responses by carbon monoxide.]

(ii) Non-human mammalian cells in vitro

No data were available to the Working Group.

4.1.6 Modulates receptor-mediated effects

(a) Exposed humans

See Table 4.10.

The modulation of receptor-mediated effects described in this section was assessed through the activation of binding to AhR and changes in circulating hormone levels associated with firefighters' exposures. Most of the studies investigated levels of hormones (namely cortisol, adrenocorticotropic hormone, catecholamines, and melatonin) related to acute exposures, and some studies investigated long-term exposures by employment as a firefighter (via levels of testosterone, thyroid function hormones, and anti-müllerian hormone). Considering the availability of the data, the studies reported below are grouped by end-point.

(i) Aryl hydrocarbon receptor

Four studies investigated AhR mediation in firefighters: one study involved a structure fire exposure (Beitel et al., 2020), and three studies considered exposures of employment as firefighter (Orris et al., 1986; Chernyak & Grassman, 2020; Ricaud et al., 2021).

Beitel et al. (2020) used a potency toxicity bioassay to evaluate AhR activation in extracts of urine and skin-wipe samples collected from firefighters before and after a fire drill, in Arizona, USA. The study included 11 firefighters; 10 firefighters participated in the training fire drill and there was one control, a by-stander in full gear, who did not enter the training building. The assay in a rat hepatoma reporter cell line transfected with a luciferase gene (polycyclic aromatic hydrocarbon-chemical activated luciferase gene expression, PAH-CALUX) measured increased agonistic response activity in samples of urine from 3 out of the 10 firefighters who participated in the fire drill. The assay response was significantly correlated with hydroxylated PAH concentrations in the urine samples, with < 1% of the bioassay response predicted by the quantified compounds excreted in the urine. The skin-wipe sample extracts showed a significant increase in AhR active compounds after firefighting compared with before firefighting, and for skin samples collected both from the neck and the calf (Beitel et al., 2020). [The Working Group noted that the observation that the urinary response exceeded the prediction for hydroxylated PAHs could be related to the urinary excretion of other compounds with AhR activity (Beitel et al., 2020), with the use of the bioassay being a strength for the analysis of complex mixtures. The Working Group further noted the small sample size of the study, particularly with high variability of the urinary excretion patterns and baseline levels, but also noted the appropriate pre-/post-exposure design. The Working Group noted that the assay response indicated that the firefighters were exposed to AhR agonists.]

Ricaud et al. (2021) investigated the potency of the AhR agonistic response in serum collected from firefighters in Montreal, Canada, and using a human liver carcinoma cell line transfected with a xenobiotic response element (XRE)–luciferase reporter gene. The firefighters were stratified by employment length (with < 10 or

Table 4.10 End-points relevant to modulation of receptor-mediated effects in exposed firefighters

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
AhR bioassay, potency toxicity assay (PAH CALUX)	Skin wipes (neck and calf) and urine	Live-fire training (burning wood pallet, furniture, carpet, and miscellaneous objects) (14 min) USA (Arizona), pre/post trial study on male firefighters before and after firefighting training activities with use of full PPE	10, 1	↑ AhR bioassay activity in skin sample extracts from post-firefighting ($P = 0.025$ for calf wipes); +, Positive correlation between bioassay response and OH-PAHs concentrations found in urine ($P = 0.008$), with < 1% of the response predicted by the quantified urinary OH-PAHs	Only non-smokers included; participants were asked to refrain from grilled food 12 h before the drill and until last urine was sampled	Small sample size with high inter-variability; the control (n =1) had unclear tasks and location Exposure assessment: biomarkers are appropriate with regards to their half-lives; potential for residual confounding by other environmental exposure, especially diet	Beitel et al. (2020)
AhR bioassay activity, potency toxicity assay (HepG2-XRE luciferase assay)	Serum	Employment as firefighter Canada, cross-sectional study on 30 male firefighters (15 with ≤ 10 yr and 15 with ≥ 20 yr of experience) and 15 healthy controls	30, 15	↑ AhR bioassay activity in firefighters compared with controls ($P < 0.05$ for firefighters ≤ 10 yr and $P < 0.01$ for firefighters ≥ 20 yr) ↑ AhR bioassay activity in firefighters hydrophobic purified fraction of sera for both groups ($P < 0.001$) and ↓ activity for all groups when added antagonist ($P < 0.05$)	Only non-smokers included; groups matched on age and sex	Exposure assessment: limited to length of employment; roles or recent exposures not described Exposure assessment: cross-sectional design with qualitative measures of exposure and potential for confounding by non-firefighting-related exposures	Ricaud et al. (2021)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
CYP1A2 activity (antipyrine metabolite excretion) and *AHRR* expression	Blood and 24-h void urine (collected in 2009–2010)	Mix (historical industrial fire and employment as firefighter in region with wildland fire events) Russian Federation, cross-sectional study in a cohort of 28 male firefighters (11 current and 17 former firefighters) and 10 controls using antipyrine as a metabolic probe; 20 out of the initial 30 firefighters were involved in a fire incident in a cable factory (in 1992), without use of respiratory protection	28, 10	CYP1A2 activity was positively associated with dioxin body burden among carriers of the *AHRR* G allele ($P = 0.04$) and associated with higher levels of *AHRR* transcript expression	Groups, matched on age and BMI, models adjusted for smoking (urinary cotinine), dioxin body burden, *AHRR* (565 > G) genotype, *AHRR* gene expression	Small sample size; included smokers Exposure assessment: appropriately used biomarker of cumulative exposure in analysis of chronic effect, especially for current firefighters	Chernyak & Grassman (2020) Complementary study, Chernyak et al. (2012)
Chloracne diagnosis	Physical examination, biopsy, and blood sample	Historical fire events and employment as firefighter USA (Illinois), case report of 2 firefighters reportedly involved in historical incidents and fires who were diagnosed with chloracne	2	The 2 cases of chloracne had historical exposures potentially consistent with the diagnosis Blood PCB levels < 10 µg/L for both cases	None	Small sample size; no controls; long lag time between possible exposure and assessment; risk of recall bias Exposure assessment: description of exposure based on self-reported participation in historical fire incidents	Orris et al. (1986)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Testosterone and cortisol (ELISA immunoassay)	Plasma (morning, 4 samples on days 1, 4, 8, and 11)	Real-fire training and physical exertion (11 days of training, including 7 in prescribed burns) USA (Montana), pre/post trial study on 16 wildland firefighters (14 men and 2 women) during critical training	16	No changes in testosterone ↑ Cortisol ($P = 0.03$) ↓ T:C ratio ($P = 0.01$)	Intra-individual	Small sample size; sequence design without control group; not possible to retrieve hormone levels segregated by sex, although time analysis was available for men Exposure assessment: engagement in the training appropriately tested as exposure in the pre/post design; exposure misclassification due to self-report of muscle soreness unlikely to affect result	Christison et al. (2021)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Testosterone (IRMA immunoassay) and cortisol (biotin-streptavidin immunoassay)	Plasma (testosterone) and saliva (cortisol) (between 9 h and 10 h, 5 blood and 30 saliva samples)	Employment as firefighter United Kingdom repeated measures in a cohort of 72 male probationary firefighters, recruited during education and followed for 1 yr, measured in 5 sessions with 3-month intervals (on the first day shift of an 8-day shift cycle)	72	↓ Testosterone from 3 to 12 months ($P < 0.001$) ↑ Cortisol from 3 to 12 months ($P < 0.03$) Session with higher daily stress were associated with lower cortisol ($P < 0.01$) and higher testosterone levels ($P < 0.025$)	Intra-individual; daily stress, anxiety, and depression inventories; control for shift work	The study included smokers, but the authors reported elsewhere a stable pattern of smoking habits and accounted for intra-individual changes; possible overlapping sample with (Roy et al., 1998; Roy, 2004) Exposure assessment: tool used to quantify job described in Roy (2004) publication attempted to account for subjectivity in reporting exposure by testing the effect of intraindividual variation in exposure measures on outcomes	Roy et al. (2003)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Testosterone (ECLIA immunoassay)	Serum (morning)	Employment as firefighter (metropolitan fire department) USA (Florida), cross-sectional study among 326 male career firefighters stratified by testosterone levels (126 borderline or low and 200 within reference levels)	126, 200	Prevalence of low and borderline testosterone levels, 37% Borderline-low testosterone associated with decreased LVWT ($P < 0.01$)	Age, BMI, SBP, and HbA1c; group with high levels ($n = 15$) eliminated from further analysis (possible supplementation)	Cross-sectional nature; population sample with large ranges for age (19–69 yr) and BMI; potential for interference of night work Exposure assessment: employment as firefighter, without further information on duration of employment, specific tasks or exposures; firefighting exposure was not quantified	Lofrano-Porto et al. (2020)
Testosterone (Access 2 immunoassay)	Whole blood (morning sample at 8 h at the start of 24-h shift, after 2 days off)	Employment as firefighter (military) Kazakhstan, cross-sectional study on 100 male military firefighters from 3 occupational subgroups: firefighters (49), fire-truck drivers (22) and management and engineers (29) and their burnout risk measured with the MBI-GS tool	100	No changes in testosterone levels per occupational group ↑ Testosterone was associated with professional efficacy burnout	Age, smoking, exercise, and health-related quality of life; by design, controlled for night shift [colinearity between age and years in service, with the latter excluded from analysis]	Cross-sectional nature; no control group; no BMI data; groups not matched on age, years in service, marital status, education, and smoking status Exposure assessment: potential for overlap in current and past overlap exposure categories (occupation)	Vinnikov et al. (2021)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Testosterone and estradiol (RIA immunoassay)	[Serum]	Employment as firefighter USA (Ohio), cross-sectional study with 12 active male firefighters (mean age, 46.2 ± 6.3 yr), used as control group, and 38 male coronary patients (admitted to hospital with acute myocardial infarction or undergoing evaluation of chest pain with or without CAD)	12, 38	↓ Estradiol for firefighters vs acute patients ($P < 0.01$) No changes in testosterone ↑ BMI in firefighters compared with patients without CAD ($P < 0.025$)	Age and BMI	Small sample size; cross-sectional nature; sample timing not reported; no comparison with healthy participants; firefighting exposure not assessed; incongruence in biosample definition in methods and table heading Exposure assessment: employment as firefighter, without further information on duration of employment, specific tasks or exposures; firefighting exposure was not quantified	Luria et al. (1982)
Testosterone and cortisol (RIA immunoassay)	Saliva (2 samples)	Stress from examination (dog handlers) and employment as firefighter USA (California), pre/post study in a disaster dog handler certification test, using 16 handlers (7 firefighters among them) and 6 evaluators	7, 9	No changes in testosterone ↓ Cortisol levels for firefighters ($P < 0.05$)	Dichotomized timing of post sample	Small sample size; controls not matched; food and caffeine intake not controlled; post-sample time span from 09:30 to 15:00; occupation not described for non-firefighters Exposure assessment: firefighting exposure was not quantified	Lit et al. (2010)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol and ACTH (RIA immunoassay)	Plasma (3 samples, [morning])	Live-fire training (17 min) USA (Illinois), pre/post trial study in male professional [career] firefighters with use of full PPE (before, immediately after and 90-min recovery of fire drill)	11	↑ACTH ($P = 0.002$) ↑ Cortisol ($P < 0.001$) and was still elevated after 90 min	Intra-individual; by design, control of food intake, and physical and thermal strain	Small sample size; sequence design without control group; reported cortisol units may be wrong Exposure assessment: appropriate in terms of assessing the effect of firefighting; no specific firefighting hazard assessed	Smith et al. (2005)
Cortisol and ACTH (CLIA immunoassay)	Serum (4 samples, before, immediately, 4 h and 24 h after exposure)	Live-fire training (40 min) Republic of Korea, pre/post trial study on firefighting instructors performing live fire suppression in training facility and firefighting instructors performing physical exercise with full PPE without ambient heat	7, 7	↑ ACTH immediately after live-fire simulation ($P < 0.05$) No changes in cortisol level among the groups, with level elevated after the live-fire simulation	None	Small sample size; physical exertion not controlled; repeated measurements (intra-individual) dependence not considered in analysis; sex and sampling timing not reported; cortisol detection method not reported Exposure assessment: involvement in controlled hot working and smoke exposure conditions appropriately tested as exposure for the effects assessments	Kim et al. (2018)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol (ELISA immunoassay)	Saliva (4 samples: morning baseline day, morning exposure day, immediately after exercise and 30 min after exercise)	Search and rescue exercise while using full PPE [live-fire training] (60 min) United Kingdom, RCT on the combined glucose and caffeine administration to participants attending a 3-day basic fire-training course; 3 groups: placebo drink, high glucose and low caffeine drink and low glucose and high caffeine drink	27, 26, 27	↑ Cortisol after exposure to fire-fighting exercise ($P = 0.019$) No changes (or difference) among groups	Control by design (matched) on age, gender, BMI, years of education and time of awakening	Exposure assessment: engagement in live-fire drill appropriately tested as exposure for the effects assessments	Sünram-Lea et al. (2012)
Cortisol (ELISA immunoassay)	Saliva (6 samples over 3 days, collected between 13:30 and 16:00	3 training days with live-fire on third day (60 min) United Kingdom, pre/post trial study on novice firefighters (men and women) over a 3-day firefighting course with morning classroom and afternoon exercises of 2 h, with increased intensity over the 3 days (live-fire only on the third day) and 11 non-firefighter control participants	21, 11	↑ Anticipatory cortisol in firefighters group ↑ Cortisol levels after live-fire firefighting for both firefighter groups (assessed immediately or after 20 min) ($P = 0.03$) No changes in cortisol levels in training sessions without live fire	By design control of awakening patterns [Mixed ANOVA accounted for intra-day variation, no intra-individual]	No information on smoking status Exposure assessment: appropriate in terms of assessing the effects of live-fire suppression	Robinson et al. (2013)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol (ELISA immunoassay)	Saliva (5 or 6 samples depending on group: 2 at baseline, 2 or 3 after evolutions, 1 at recovery and 1 at completion of protocol)	Live-fire training (wood fire) (30 or 45 min) USA (Pennsylvania), pre/post trial study to examine the influence of workload duration of experienced firefighters (mean age, 30.3 ± 8.3 yr) engaged in fire suppression; randomized groups: 2 or 3 bouts of fire suppression activity	42	No difference in cortisol output was found between the groups ↓ Cortisol over the course of the live-fire evolution in both groups ($P < 0.05$)	Intra-individual	Men and women included; difficulties in controlling length of exercises; loss of samples due to reduced saliva at later time-points; staggered experiment start times and cortisol samples; high anticipatory (baseline) levels Exposure assessment: appropriate in terms of assessing the effects workload suppression training	Rosalky et al. (2017)
Cortisol (CLIA immunoassay for serum samples and LC-MS/MS for saliva samples)	Serum and saliva simultaneously sampled 3 times (1 h before, immediately after and 10 h after the simulation training)	Simulated terrorist attack (shooting, hostage and live-fire in parked cars) (2 h) Netherlands, pre/post trial on first responders before and after a simulated emergency exercise; participants included 5 different groups of first responders including firefighters	10 firefighters, 26 other first responders (ambulance crew, emergency department, police officers, rapid response team) and 34 observers used as control group	↑ Cortisol levels among first responders 1 h after the training ($P < 0.05$) No changes between the first responder groups	None	Repeated measurements (intra-individual) dependence not considered in time-dependence analysis; age and gender not matched between groups; groups with small sample sizes	Smeets et al. (2021)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol (ELISA immunoassay)	Saliva (5 samples: 2 samples on resting day at 7:00 and between 17:00 and 18:30, and 3 samples in intervention session, before, 30 min and 90 min after intervention)	Physical exertion while using full PPE (total weight of ensemble was 23 kg, no fire involved) (12 min) Italy, pre/post trial study on male firefighters (mean age, 32 ± 1 yr) to investigate the effect of firefighting simulation exercise (climb ladder and descend carrying dummy, run, complete a maze in the dark and run again)	20	↑ Cortisol levels 30 min after intervention ($P < 0.001$), with return to baseline after 90 min	Intra-individual	Sequence design Exposure assessment: physical exertion was assessed using a simulated rescue intervention	Perroni et al. (2009)
Cortisol (ELISA immunoassay)	Saliva (morning, 3 samples per session)	Simulated fire-grounds test (9 firefighter-specific tasks, no fire) while wearing full PPE and SCBA (7–10 min) USA (Texas), pre/post trial on 13 professional [career] male firefighters challenged in a firegrounds test after an 8-wk time period under a TRF protocol (14 h fasting:10 h feeding); saliva sampling before, immediately and 30 min after the test	13	↑ Cortisol concentrations pre and 30 min post firefighting simulation test following TRF ($P < 0.05$) ↓ Cortisol concentrations immediately after firefighting simulation test following TRF ($P < 0.05$)	Intra-individual	Small sample size; sequence design; no report or control of firefighting duties before sessions Exposure assessment: engagement in experimental fitness test appropriately tested as exposure for the effects assessments that were done in the experiment	McAllister et al. (2021)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol (ELISA immunoassay)	Saliva (total of 10 samples per subject, with 5 samples per boot-type-session: baseline, immediately after 2 trials and 30 min after second trial)	Simulated stair climb (2 trials of 3 min per boot-type-session, no fire) USA (Mississippi), pre/post trial to examine the physiological difference between 2 boot types (rubber boots and leather boots) used while performing a simulated stair climb wearing full firefighting equipment	12	↑ Cortisol levels when using leather boots ($P < 0.05$) No correlation between cortisol and variables of leg strength	Intra-individual; counterbalanced order of testing	Small sample size Exposure assessment: engagement in experimental stair climb exercise appropriately tested as exposure	Huang et al. (2009)
PGC-1α, NE and EPI (ELISA immunoassay) ACTH, PTH and insulin (Luminex multiplex immunoassay)	Plasma (5 samples)	Physical exertion (and employment as firefighter, no fire) Spain, RCT on 2 weeks ubiquinol supplementation on 100 male firefighters	50, 50 [some lost in follow-up, being 34–34 for the last assessment]	↑ PGC-1α with exercise and higher in ubiquinol group ↑ ACTH with exercise, no effect on ubiquinol ↑ EPI and NE with exercise ($P < 0.05$) ↑ NE with ubiquinol ($P < 0.05$) ↓ Insulin with exercise ↑ PTH in ubiquinol group	Smoking, self-reported information on diet and physical activity	Blood sampling day-timings not reported; firefighters used as a convenience group without control for occupational activity Exposure assessment: engagement in experimental physical exercise appropriately tested as exposure in the RCT design to test an intervention	Diaz-Castro et al. (2020b)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
NE and EPI (HPLC-ECD)	Plasma (11 samples)	Physical exercise and mental challenge (no fire) USA (Mississippi), pre/post trial on experienced male firefighters performing simulated exercise with or without simultaneously being challenged with a computerized firefighting strategy and tactics drill	9	↑ NE and EPI after challenge, with greater increase after dual challenge (physical and mental) +, NE was correlated with IL-2 in dual challenge	Intra-individual	Small sample size; possible overlapping sample with Webb et al. (2011) Exposure assessment: engagement in experimental drill exercise appropriately tested as exposure for the effects assessments that were done in the experiment	Huang et al. (2010a)
Cortisol (RIA immunoassay), ACTH (IRMA immunoassay), NE and EPI (HPLC-ECD)	Plasma (11 samples)	Physical exercise and mental challenge (no fire) USA (Mississippi), pre/post trial on experienced male firefighters performing simulated exercise with or without simultaneously being challenged with a computerized firefighting strategy and tactics drill	12	↑ Cortisol after dual challenge ↑ NE and EPI after challenge, with greater increase after dual challenge No change in ACTH for condition or time	Intra-individual	Small sample size; reported catecholamine units may be wrong; possible overlapping sample with Huang et al. (2010a) Exposure assessment: engagement in experimental drill exercise or mental challenge appropriately tested as exposure for the effects assessments	Webb et al. (2011)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol (ELISA immunoassay) and relationship with cytokines (Milliplex MAP human cytokine immunoassay)	Saliva (cortisol, 9 samples) and plasma (cytokines, 4 samples)	Simulated physical demands involved in wildfire suppression and sleep restriction (no ambient heat or smoke) Australia, pre/post trial study in deployed firefighters (30 men and 5 women) during 3 days performing simulated occupational physical demands with or without sleep restriction	17, 18	↑ Morning IL-6 related to ↑ evening cortisol in sleep restriction group, while in control group a ↑ IL-6 was associated with a ↓ in evening cortisol	Intra-individual (additionally sex, age, and BMI); control for fluid consumption; matched groups	Small sample size; no crossover condition Exposure assessment: longer sleep opportunity does not automatically result in more sleep; authors did present the actual hours slept, which was significantly different between groups	Wolkow et al. (2015b)
Cortisol (ELISA immunoassay) and relationship with cytokines (Milliplex MAP human cytokine immunoassay)	Saliva (cortisol, 8 samples) and plasma (cytokines, 4 samples)	Simulated physical demands and ambient temperature Australia, pre/post trial study in deployed firefighters during 3 days performing simulated occupational physical demands involved in wildfire suppression in mild or hot ambient temperature condition	19, 18	↑ Cortisol across time-points, independent of condition ($P < 0.001$) ↑ Morning IL-6 related to elevated cortisol independent of condition ($P < 0.024$)	Intra-individual; matched groups	Small sample size; no crossover conditions Exposure assessment: exposure to 2 different temperatures appropriately tested as exposure for the effects assessments that were done in the experiment	Wolkow et al. (2017)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol (biotin-streptavidin immunoassay with TR-FIA)	Saliva (8 samples from 10 h to 12 h)	Mental challenge (arithmetic task and speech task, no fire) United Kingdom, pre/post trial study on probationary male firefighters before and after mental challenge tasks by smoking status	86 (52 non-smokers and 34 smokers, with 19 moderate and 15 heavy smokers)	↑ Cortisol after mental challenge among non-smokers	Intra-individual; stable pattern of smoking habits; groups were comparable in terms of alcohol consumption, exercise levels, life events, daily stress and social support, psychological characteristic, but not for body weight (lower in smokers)	Overlapping sample with Roy (2004)	Roy et al. (1994)
Cortisol (biotin-streptavidin immunoassay with TR-FIA)	Saliva (7 samples between 10 h and 12 h)	Mental challenge (arithmetic task and speech task, no fire) United Kingdom, pre/post trial study on probationary male firefighters before and after mental challenge, and association with prior life events and social support	90	↑ Cortisol levels after mental challenge tasks No difference between high or low social-support groups	Intra-individual; no significant differences in smoking status among groups	No control for smoking habits; possible overlapping sample with Roy et al. (2003); Roy (2004)	Roy et al. (1998)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol (biotin-streptavidin immunoassay with TR-FIA)	Saliva (8 samples beginning between 9 h and 10 h)	Mental challenge (arithmetic task and speech task, no fire) United Kingdom, pre/post trial study on probationary male firefighters before and after mental challenge tasks, within 1 month of participants joining their fire station; sessions were arranged for the first day of the 8-day shift cycle (2 days, 2 nights and 4 days off)	82	↑ Cortisol levels after mental challenge tasks	Intra-individual	No control for smoking habits; possible overlapping sample with Roy et al. (1998, 2003) Exposure assessment: tool used to quantify job attempted to account for subjectivity in reporting exposure by testing the effect of intraindividual variation in exposure measures on outcomes	Roy (2004)
Cortisol (RIA immunoassay)	Saliva (4 samples for cortisol awakening response and 5 samples in the afternoon after exposure assessment)	Use of protective mask, no fire Switzerland, pre/post trial study on male recruits from the ERS of the Swiss Army, used as controls to male army recruits having a fear of wearing protective mask, assessed before and after cognitive-behavioural treatment	39, 46	↓ Cortisol for ERS recruits (morning levels as well as initial and final levels after mask use sessions) ($P < 0.05$)	Control of age by design	The ERS recruits were compared with a group suffering use of mask phobia Exposure assessment: appropriate design comparing pre and post levels among participants with condition of interest to the general control; condition of interest was self-reported	Brand et al. (2011)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol (biotin-streptavidin immunoassay with TR-FIA)	Saliva (morning sample)	Employment as firefighter [municipal] United Kingdom, cross-sectional study on the associations of morning cortisol and social desirability scores among firefighters (mean experience, 15.2 yr), stratified by age group	85	+, Morning cortisol was correlated with social desirability scores for firefighters under age 45 yr ($n = 60$, $P = 0.03$) but not for all samples ($n = 85$) or for age > 45 yr ($n = 25$)	None	Cross-sectional nature; 1 single sampling; no control group	Brody et al. (2000)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol (RIA immunoassay)	Saliva (sampled between 2000–2002)	Occupational participation in a major historical air disaster Netherlands, cross-sectional study on cortisol associations with PTSD and NLE established after a major air disaster in 1992	1082, 798	Exposure to the air disaster was not associated with cortisol +, Exposed participants who self-reported more intrusion symptoms had lower cortisol levels ($P < 0.05$)	Salivary sampling time, age, gender, and smoking status	Cross-sectional nature; no control for food and coffee intake, and cigarette use; large salivary sampling time span (09:00 to 16:30); not possible to retrieve results from firefighters among the study population; incongruences in numbers of excluded participants described in text and tables Exposure assessment: sample population included firefighters and police but relationships between exposure of interest and outcome were not analysed according to the occupation, which is a potentially relevant exposure metric; the 8-yr criterion for dichotomization of NLE was not justified	Witteveen et al. (2010)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Cortisol (RIA immunoassay)	Saliva (4 samples during 1 working day at 7:00, 11:00, 15:00, and 22:00)	Employment as firefighter (day without emergency situation) Czechia, repeated measurements on 136 male firefighters and 40 male and 102 female primary school teachers; firefighters were asked to perform sample collection on a day without emergency situations and teachers during their busiest workday	136, 142	↓ Cortisol (diurnal slope, morning, evening, and hormonal output) for male firefighters ($P = 0.042$)	Gender, age, physical activity, and smoking status	Cross-sectional nature; no control day measurements; age, work experience, marital status and education level not matched between groups	Susoliakova et al. (2014)
Cortisol (CLIA immunoassay)	Serum and urine (blood at 09:00, urine from 22:00 to 07:00, multiple samples)	Work shift organization (routine work) Republic of Korea, pre/post trial; repeated measurements on 325 firefighters (303 men and 22 women), including routine jobs of fire suppression, emergency medical service, rescue and fire investigation, with different work shift cycle schedules (3-, 6-, 9- or 21-day cycles)	325	↑ Serum cortisol levels after night or 24-h shift and different for different schedules; recovery of urine cortisol was delayed for those working on 6- and 21-day cycles	Sex, age, chronotype, depression, job, PTSD, sleep disorder, fatigue, caffeine, subjective health condition and sleep quality	Workload not controlled Exposure assessment: adequate exposure assessment using apparent work-shift categories for the effect that is being assessed	Lim et al. (2020)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
TSH and total T4 (ELISA immunoassay)	Plasma (collected in 2014–2015)	Employment as firefighter (years of work, on duty shift, firefighting in the last 24 h or 7 days, use of SCBA, job function) USA (California), cross-sectional study on associations between urinary excretion of metabolites of flame retardants and thyroid function among women firefighters compared with office workers	84, 81	↑ BDCPP in firefighters and associated with a T4 decrease ($P < 0.05$)	Age and creatinine; by design control of medication; food consumption not associated with metabolite excretion for either group	Cross-sectional nature; exposure markers analysed in spot urine samples Exposure assessment: although creatinine-corrected, spot urine was used for this cross-sectional study; levels may be impacted by non-work sources	Trowbridge et al. (2022)
TSH, unbound T4 and T3	Blood (2 samples, baseline and week 52) (2019–2021)	Employment as firefighter Australia, randomized clinical trial examining the effect of plasma and whole blood regular donation on PFAS blood levels and thyroid function on firefighters with baseline PFOS level ≥ 5 ng/mL	285	Plasma and blood donation decreased significantly PFAS levels, and plasma donation had a larger treatment effect than blood donation; unchanged levels of thyroid function hormones; group-screening level interactions for low and high levels of TSH (with plasma donation associated with larger increase of TSH for higher baseline TSH)	Intra-individual (mean change)	Thyroid function hormone detection method not reported Exposure assessment: exposure (whole blood vs plasma donations vs no donations) was controlled for in the design of the clinical trial	Gasiorowski et al. (2022)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
AMH (picoAMH ELISA)	Dried blood spots	Employment as firefighter USA (Arizona) and Canada, cross-sectional study on association of AMH and firefighting occupation among 106 female firefighters and 58 non-firefighter female controls.	106, 58	↓ 33.4% (95% CI, −55.0 to −0.14) AMF in firefighters Among firefighters, no change in AMH for number of live fires responded to in a typical month or years worked in the fire service	Age and BMI; only non-smokers included	No information on non-firefighters' occupation Exposure assessment: it was qualitative as history of firefighting; use of PPE was accounted for	Davidson et al. (2022)

Table 4.10 (continued)

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Melatonin (ELISA immunoassay)	Saliva (every 4 h during night shift, 4 samples)	Night work shift organization (routine work at petrochemical plant) Islamic Republic of Iran; repeated measurements on firefighters at a petrochemical plant following 2 different night shift work plans (7 or 4 consecutive night shifts)	64	Melatonin night rhythm was different among the 2 work shift cycles ($P < 0.001$)	Participants were asked to keep regular sleep schedules and avoid eating 1 h before sampling; models adjusted for light exposure and caffeine consumption	Melatonin rhythm only assessed in the last night of both shift cycles (with different cycle lengths), not assessed during the day or day shifts; cross-sectional nature; incongruent reporting of group demographic differences; caffeine assessment method not described; inconsistent description of how many participants lived far from their families Exposure assessment: it was accurate; participants were selected on the basis of apparent work shift categories	Kazemi et al. (2018)

ACTH, adrenocorticotropic hormone; AMH, anti-müllerian hormone; BDCPP, bis(1,3-dichloro-2-propyl)phosphate; BMI, body mass index; CAD, coronary artery disease; CALUX, chemical activated luciferase gene expression; CLIA, chemiluminescence immunoassay; CYP1A2, cytochrome P450 1A2; ECLIA, electrochemiluminescence assay; ELISA, enzyme-linked immunosorbent assay; EPI, epinephrine; ERS, emergency rescue service; HbA1c, glycosylated haemoglobin; HPLC-ECD, high performance liquid chromatography-electrochemical detection; IL-2, interleukin 2; IQR, inter-quartile range; IRMA, immunoradiometric assay; LVWT, left ventricular wall thickness; MBI-GS, Maslach Burnout Inventory-General Survey; NE, norepinephrine; NLE, negative life events; OH-PAHs, hydroxylated PAHs; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFOS, perfluorooctanesulfonic acid; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1 α; PPE, personal protective equipment; PTH, parathyroid hormone; PTSD, post-traumatic stress disorder; RCT, randomized controlled trial; RIA, radioimmunoassay; SBP, systolic blood pressure; SCBA, self-contained breathing apparatus; T4, thyroxine; T:C, testosterone:cortisol ratio; TRF, time-restricted feeding; TR-FIA, time-resolved fluorometric end-point determination; TSH, thyroid-stimulating hormone; XRE, xenobiotic response element; vs, versus; yr, year.

[a] +, positive; –, negative; +/–, equivocal; (+) or (–), positive or negative result in a study of limited quality; ↑, increase; ↓, decrease.

[b] Factors to be considered for study quality included the methodology and design, reporting, and exposure assessment quality.

> 20 years of experience) and compared with an age- and sex-matched control group of healthy non-firefighters. The bioassay activity increased significantly when the transfected cells were treated with heat-inactivated serum from either firefighter group compared with the control group but was not different between the two groups of firefighters. [The Working Group noted that recent exposures or the different roles that firefighters assume may have the potential to affect the ability to distinguish between firefighter employment length.] Significant AhR activity was also reported when the bioassay was treated with purified hydrophobic fraction of firefighters' sera. A ligand–receptor interaction was confirmed by a significant decrease in the bioassay activity, for all groups, when an AhR antagonist (GNF351) was added to the purified serum fraction. [The Working Group deemed this an informative study because of the investigation of heat-inactivated serum, purified fraction of serum, and confirmation of an antagonistic effect, and the inclusion of solely non-smoking, male, and age-matched participants, who were compared with a control group of non-firefighters.]

Chernyak & Grassman (2020) investigated the effect of the AhR repressor (*AHRR*) polymorphism (565C > G or Pro185Ala, rs2292596) on the activity of hepatic enzyme cytochrome P450 1A2 (CYP1A2), a downstream target of AhR, in blood samples from 28 male firefighters (former and current) and 10 matched male non-firefighter controls. The firefighters were recruited from a cohort established after an historical industrial fire in a cable factory in Shelekhov, Russian Federation, which they had attended without use of respiratory protection; samples were collected 17 years after the incident. CYP1A2 activity, assessed in urine using antipyrine as a metabolite probe, was positively associated with dioxin body burden among carriers of the *AHRR* G allele (Chernyak & Grassman, 2020). The study indicated that the variant alanine (GG and GC) exhibited stronger AhR repression than did the CC genotype, determined as higher gene expression of *AHRR* and lower activity of CYP1A2. The models using current firefighters showed the best fit, with dioxin body burden being significantly associated with CYP1A2 activity when adjusting for *AHRR* genotype. In a previous study from the same group using the same participant samples, the authors reported higher levels of dioxin-like compounds in firefighters than in non-firefighters and higher levels of PCBs among current firefighters (Chernyak et al., 2012). [The Working Group noted that the study demonstrated an association between the toxicant body burden and the level of activity of enzymes involved in its biotransformation, mediated by the *AHRR* genotype.]

Two firefighters from Chicago, Illinois, USA, were reported with a diagnosis of chloracne relating to possible historical occupational exposures (Orris et al., 1986). Each case reported 23 years or 20 years of employment as a firefighter and participation in possible historical events, with 10 years and 15 years, respectively, since onset of symptoms. At the time of diagnosis, blood levels of PCBs were < 10 µg/L. [The Working Group noted that although the temporal relationship between possible occupational exposures and onset of symptoms might be plausible, for a disease mediated by AhR, no definitive etiological relationship could be established.]

[The Working Group noted that, overall, the three available studies (Beitel et al., 2020; Chernyak & Grassman, 2020; Ricaud et al., 2021) and the case report (Orris et al., 1986) all demonstrated AhR activation, measured through various end-points, with firefighting exposures. Although the studies were limited by small sample sizes and risk of recall bias (for the case report), collectively they pointed to agonistic binding and activation effects.]

(ii) Androgens and estrogens

Six studies investigated the levels of sex hormones in firefighters: one study was related to wildland critical training in prescribed burns (Christison et al., 2021), and five studies considered employment as a firefighter (Luria et al., 1982; Roy et al., 2003; Lit et al., 2010; Lofrano-Porto et al., 2020; Vinnikov et al., 2021), with two of these studies also investigating exposure to stress (Roy et al., 2003; Lit et al., 2010).

Christison et al. did not detect differences in morning plasma testosterone levels in 14 male and 2 female firefighters over 11 days of critical training with 7 days on prescribed burns, in Montana, USA. They reported a decreased testosterone:cortisol ratio after day 8; this is a marker for overreaching, which was correlated with muscle damage and soreness (Christison et al., 2021).

A cohort of 72 male probationary firefighters, from London, United Kingdom, was followed over 1 year, measured in five sessions, to investigate the within-individual relationship between recent stress exposure and testosterone levels (Roy et al., 2003). The five repeated session measurements were performed in the same place, same time of the day, and on the same day of the shift cycle, at 3-month intervals across the year. A decrease in morning plasma testosterone levels across the assessment sessions was observed, with higher prior stress associated with higher testosterone levels, whereas there was an increase in salivary cortisol levels (described below in Section 4.1.6(a)(iii) (Roy et al., 2003). [The Working Group noted that the observations suggested glucocorticoid-mediated testosterone suppression. The Working Group considered this study to be informative because of the repeated measurement design and use of probationary firefighters without previous firefighting exposures, adequate follow-up duration, reasonable sample size, with control for shift work, sampling timing, and (although including smokers) for smoking habits and intra-individual changes.]

Three cross-sectional studies reported total testosterone levels in male firefighters. Lofrano-Porto et al. (2020) reported a prevalence of 37% for low and borderline serum testosterone levels among 326 male career firefighters (stratified by reference values), from Florida, USA; this was associated with decreased left ventricular wall thickness. [The Working Group noted that the group with low testosterone levels was significantly older and had a higher body mass index (BMI) than did the group with testosterone levels that were within the reference range, whereas the group with borderline testosterone levels had a significantly lower age and BMI than did the group with low testosterone levels.] However, Vinnikov et al. (2021) reported normal blood testosterone levels for all 100 military firefighters, from Kazakhstan, from three occupational groups (firefighters, fire-truck drivers, and management and engineers), and no difference between firefighter groups, with higher testosterone levels associated with burnout risk as assessed by an inventory validated tool. [The Working Group noted that the groups were not matched for age or years in service, BMI data was not reported, and there was no non-firefighter control comparison.] Another cross-sectional study with 12 male firefighters as a healthy control group, from Ohio, USA, reported lower serum estradiol levels in firefighters than in male patients with acute infarction, and no difference between firefighters and male patients undergoing evaluation of chest pain with or without evidence of coronary artery disease (Luria et al., 1982). Additionally, BMI was significantly higher in firefighters than in the patients without notable coronary obstruction, and no differences were reported in BMI and age-adjusted total serum testosterone levels between the groups (Luria et al., 1982). [The Working Group noted that the comparison was limited to disease status (which may lead to uncertainties in the interpretation of

the results), used a small sample size, and had no exposure assessment.]

There was also no difference detected in saliva testosterone levels in seven firefighters from California, USA, sampled before and after a stress challenge (Lit et al., 2010). [The Working Group noted the sampling time span and small sample size.]

In total, six studies investigated testosterone levels in firefighters: two studies showed effects (Roy et al., 2003; Lofrano-Porto et al., 2020) and four studies showed unchanged levels (Luria et al., 1982; Lit et al., 2010; Christison et al., 2021; Vinnikov et al., 2021). [The Working Group noted that the studies with no effects were less informative, because of small sample sizes, lack of a control group, or non-matched or non-adequate sampling timings.]

(iii) Cortisol, adrenocorticotropic hormone, and catecholamines

Of seven studies investigating cortisol levels in scenarios involving live-fire drills, six studies reported increased cortisol levels (Smith et al., 2005; Sünram-Lea et al., 2012; Robinson et al., 2013; Kim et al., 2018; Christison et al., 2021; Smeets et al., 2021), with only one study reporting that levels were not significantly affected (Rosalky et al., 2017). [The Working Group noted that the staggered experiment start times, possible elevated anticipatory levels, loss of post-exposure samples, and difficulty in controlling the length of the exercise might have precluded the ability to observe effects in Rosalky et al. (2017).]

In firefighters (n = 325) from Republic of Korea, following four different night shift cycles, morning serum cortisol levels were higher after working a night shift than after working a day shift. (Lim et al., 2020).

Roy et al. (2003) observed (together with the testosterone decrease reported earlier in Section 4.1.6(a)(ii)) increased salivary cortisol levels after 1 year of follow-up of probationary firefighters. Sessions with higher daily stress before the assessment were associated with lower cortisol levels, suggesting downregulation of cortisol following an increment in stress exposure (Roy et al., 2003).

Cortisol levels also increased after physical exertion simulations in six studies without live fires in Australia, Italy, and the USA (Huang et al., 2009; Perroni et al., 2009; Webb et al., 2011; Wolkow et al., 2015b, 2017; McAllister et al., 2021). McAllister et al. investigated a time-restriction feeding regime and reported a shift in cortisol response and changes in inflammation markers among 13 firefighters following a simulated fire-ground challenge (McAllister et al., 2021). Wolkow et al. investigated the dual challenge of physical work and sleep restriction. Firefighters undertaking 3 days of physical work with 2 nights of sleep restriction had increased levels of salivary cortisol when compared with firefighters with 8 hours of sleep opportunity. Increased morning interleukin IL-6 levels in plasma were related to increased evening levels of salivary cortisol in the sleep-restricted group and decreased evening cortisol levels in the control group (Wolkow et al., 2015b). The authors reported that subjective self-reported mood and physical signs and symptoms were also related to cortisol levels (Wolkow et al., 2016a, b). In a study with a similar deployment design but for a dual challenge of physical exercise and hot ambient temperature, increases in cortisol and plasma IL-6 levels were observed, independently of conditions, suggesting that there was no effect of ambient temperature (Wolkow et al., 2017).

Mental stress alone was observed to increase cortisol levels in a pre/post trial (Roy et al., 1994, 1998; Roy, 2004), and two studies reported lower levels of cortisol after stress in firefighters than in control groups assigned to different tasks (Lit et al., 2010; Brand et al., 2011). [The Working Group noted that these studies were not informative because the comparison was only made with participants having a phobia (Brand et al., 2011) or because of small sample size and study design

(Lit et al., 2010).] Salivary cortisol levels associated with self-reported stress indicators were also observed in cross-sectional studies (Brody et al., 2000; Witteveen et al., 2010). [The Working Group noted that the study by Witteveen et al. (2010) presented limitations because of the saliva sampling design.] Repeated measurements in 136 firefighters and 142 primary school teachers showed lower morning, evening, and diurnal slope salivary cortisol levels, with overall cortisol output being lower in male firefighters than in male teachers (Susoliakova et al., 2014). [The Working Group noted that the groups were not matched, and mental stressors were not controlled for – firefighters were sampled on a day without an emergency call and teachers were sampled on their busiest day.]

The effect of dual challenge with physical and mental stress from a firefighting simulation exercise showed increased plasma cortisol levels, together with increased epinephrine and norepinephrine, after the dual challenge in comparison with physical exercise alone (Huang et al., 2010a; Webb et al., 2011).

Adrenocorticotropic hormone and catecholamines, which are less well-studied than cortisol, were also observed to be affected by live-fire training (Smith et al., 2005; Kim et al., 2018) and physical exercise (Diaz-Castro et al., 2020b), or by dual challenge (Huang et al., 2010a; Webb et al., 2011).

(iv) Peroxisome proliferator-activated receptor γ coactivator-1α, parathyroid hormone, thyroid-stimulating hormone, thyroxine, anti-müllerian hormone, and melatonin

A controlled trial of ubiquinol supplementation in a sample of 100 firefighters also reported increased levels of plasma peroxisome proliferatoractivated receptor γ coactivator-1α (PCG-1α), and parathyroid hormone, both after the physical challenge protocol and as an effect in the ubiquinol-supplemented group (Diaz-Castro et al., 2020b). [The Working Group noted that the study did not control for firefighters' occupational activity, and the physical challenge test may not have been representative of firefighters' physical exertion exposure.]

Trowbridge et al. investigated the associations between urinary excretion of flame retardant metabolites and plasma levels of thyroid-stimulating hormone (TSH) and thyroxine (T4) in a cross-sectional study comparing 84 female firefighters with 81 female office workers from the San Francisco Fire Department, USA. The authors observed a relationship between levels of flame retardant metabolites and T4 but not TSH: levels of bis(1,3-dichloro-2-propyl) phosphate (BDCPP) among firefighters were two-fold those among office workers and were associated with decreased T4 levels; this association was not observed among office workers (Trowbridge et al., 2022).

A randomized control trial involving 285 firefighters investigated the effects of repeated donations of plasma and blood on levels of PFAS and thyroid function hormones (Gasiorowski et al., 2022). The firefighters (current or former) with baseline PFOS levels of ≥ 5 ng/mL were assigned to repeatedly donate plasma or blood, or to be observed for 1 year. Plasma and blood donation both significantly decreased PFOS levels in firefighters compared with the observation-only group, and plasma donation had a larger treatment effect than did blood donation, but thyroid function (as measured by levels of TSH, triiodothyronine T3, and T4) was unchanged 1 year after repeated donations, compared with baseline (Gasiorowski et al., 2022).

The association between the occupation of firefighter and levels of anti-müllerian hormone, a clinical marker of ovarian reserve used to assess responsiveness to fertility treatment, was investigated in a cross-sectional study involving 106 female firefighters and 58 female non-firefighter controls (Davidson et al., 2022). Firefighters had lower levels of anti-müllerian hormone than did non-firefighters.

Kazemi et al. investigated salivary melatonin levels and self-reported sleepiness among firefighters at a petrochemical plant in the Islamic Republic of Iran who were following two different night shift cycles: 4 nights, 4 days, and 4 days off (rest days); or 7 nights, 7 days, and 7 days off. The melatonin circadian rhythm at night of firefighters showed a delayed peak in the last night of the 7-night shift and was associated with a delayed peak in sleepiness (Kazemi et al., 2018). [The Working Group noted that melatonin rhythm was only assessed in the last night of both shift cycles, with different lengths, and that changes may have been an adaptation to the night shift.]

(b) Human cells in vitro

No data were available to the Working Group.

(c) Experimental systems

(i) Non-human mammals in vivo

No data were available to the Working Group.

(ii) Non-human mammalian cells in vitro

One study evaluated estrogenic activity in extracts of firefighters' gloves and hoods in an estrogen screening assay in yeast; estrogenic and anti-estrogenic activity was measured in new and used gear; the outer layer of new gloves showed estrogen activity comparable to that of 1 nM estradiol (Stevenson et al., 2015; Table 4.11) [The Working Group noted that few samples of gear were analysed, and there was no information on characteristics of the equipment.]

Behnisch et al. investigated the thyroid hormone-disrupting effects of PFAS in technical mixtures of aqueous film-forming foams (AFFFs) using a cell reporter bioassay with a thyroid transporter transthyretin construct (TTR TRβ CALUX). The three AFFF mixtures tested showed thyroid disruptive potential, both with and without total oxidizable precursor treatment (for complete oxidation of precursors); higher activities were reported for the older AFFFs from 2013 than for AFFFs from 2019 (Behnisch et al., 2021; Table 4.11). [The Working Group noted that the AFFF samples constituted technical mixtures and not the foam itself, with unknown potential exposure concentrations, and were nevertheless tested at a dilution of 100 or 10 000 times.]

4.1.7 Evidence relevant to other key characteristics of carcinogens

(a) Causes immortalization

See Table 4.12.

Telomere length is an established marker of health and disease; reduced telomere length is observed with ageing, and increased telomere length is observed in malignant cells as part of the immortalization process in some cancers (Lansdorp, 2022). In terms of markers of cellular immortalization, only two epidemiological studies were available that assessed telomere length in samples from firefighters or firefighters in training, including one study that also conducted an assessment in vitro (Ma et al., 2020; Clarity et al., 2021).

(i) Exposed humans

Ma et al. (2020) examined the short-term impact of exposure to smoke from training fires on telomere length by comparing three samples from non-smoking conscripts attending a 3-day smoke-diving training course in Denmark. No statistically significant differences were reported in telomere length between sampling time-points (14 days before the training exercise, and immediately after and 7–14 days after the training exercise).

Clarity et al. (2021) assessed telomere length in 84 female firefighters who had worked for ≥ 5 years in California, USA, and in 79 female office workers. In this cross-sectional study, serum levels of 12 PFAS and urinary levels of 10 organophosphate flame retardants were quantified in both groups, and associations between widely

Table 4.11 End-points relevant to modulation of receptor-mediated effects in experimental systems in vitro

End-point	Test system	Detection	Positive control	Sample	Estrogenic activity (significance)[a]	Comments	Reference
Estrogenic activity (YES assay)	Yeast (engineered BJ2168 strain)	Luminescence assay (estrogenic activity) and haematocytometer (anti-estrogenic activity)	17β-estradiol	Extracts from firefighter gloves and hoods (new and with 8 wk use)	+, Hoods and outer and middle layers of new gloves with estrogenic activity ($P < 0.01$) +, Used gloves and hoods displayed low estrogenic and suggested stronger antiestrogenic activity ($P < 0.05$)	Few samples of gear analysed (1 new and 2 or 3 used); no information about characteristics of equipment	Stevenson et al. (2015)
Thyroid disruptive potential (TTR TRβ CALUX assay)	Human bone osteosarcoma epithelial cells (U2OS line) transfected with TRβ and luciferase reporter construct and combined with TTR-binding assay	Luminescence	PFOA	3 technical AFFF surfactant products from 2 different production years (2013 and 2019), tested with and without total oxidizable precursor treatment (all in triplicates)	+, All tested AFFF samples showed thyroid disruptive potential +, AFFF samples from 2013 showed higher assay activity than did samples from 2019	AFFF samples are technical mixtures and not the foam itself, nevertheless, they were diluted 100 or 10 000 times	Behnisch et al. (2021)

AFFF, aqueous film-forming foams; CALUX, chemical activated luciferase gene expression; PFOA, perfluorooctanoic acid; TRβ, thyroid receptor beta; TTR, thyroid hormone transporter transthyretin; YES, yeast estrogen screening.

[a] +, positive.

Table 4.12 End-points relevant to immortalization in exposed firefighters

End-point	Biosample, tissue, or cell type	Type of exposure, location, setting, study design	No. of exposed and controls	Response (significant)[a]	Covariates controlled	Comments[b]	Reference
Telomere length	PBMC	Exposure at firefighter training Denmark, pre/post training of smoke diving course	53 conscripts in training, sampled 3 times, before and after a 3-day smoke diving course	No changes	Sex, age, random effect for individual	Study has in vitro component that reports shorter telomere length in human cells exposed to PM; participants served as their own controls; small samples of 41 men, 12 women Exposure assessment: involvement in firefighter training tested as exposure appropriate for the effects assessments that were done in the pre/post study	Ma et al. (2020)
Telomere length	Peripheral blood	Employment as firefighter and specific chemicals California, USA, 2014–2015 Women Workers Biomonitoring Initiative, cross-sectional	84 firefighters, 79 office workers, all women	Positive association between PFAS (PFOS, PFOA, PFNA, PFDA) and ↑ telomere length; association between OPFR (BCEP) and ↓ telomere length	Age, dairy and egg consumption, urinary creatinine (varies by model)	Associations reported are when adjusting for age alone; associations were attenuated when adding additional covariates for all except PFOA Exposure assessment: chronic biomarkers PFAS and PBDEs appropriate for chronic outcome that was investigated; biomonitoring for short half-life OPFRs subject to confounding from other exposures	Clarity et al. (2021)

BCEtP, bis-2-chloroethyl phosphate; OPFRs, organophosphate flame retardants; PBMC, peripheral blood mononuclear cells; PFAS, per- and poly-fluoroalkyl substances; PFDA, perfluorodecanoic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.

[a] +, statistically significant result(s) reported; no changes, no statistically significant results reported for any end-points of interest; (+), statistically significant result but study was of limited quality; ↑, increase; ↓, decrease.

[b] Factors to be considered for study quality included the methodology and design, reporting, and exposure assessment quality.

detected exposures (> 70%) and telomere length were examined in all participants and separately by occupational group. In general, the firefighters had longer telomeres than did the office workers. Among firefighters, levels of four PFAS (perfluorodecanoic acid, PFDA; perfluorononanoic acid, PFNA; perfluorooctanoic acid, PFOA; and perfluorooctanesulfonic acid, PFOS) were significantly associated with increased telomere length after adjusting for age; only the association for PFOA remained statistically significant after adjusting for additional confounders. One organophosphate flame retardant (bis-2-chloroethyl phosphate, BCEtP) was inversely associated with telomere length among firefighters. [The Working Group noted that strengths of the study included measurement of multiple exposure biomarkers in firefighters and in the control group. Limitations included lack of certainty that exposures were from the occupation and not from another source.]

[The Working Group noted that the differences in the two studies may be attributed, in part, to differences in the focal exposures – acute exposure to fire smoke during training versus chronic exposures to PFAS and organophosphate flame retardants.]

(ii) Human cells in vitro

Ma et al. (2020) treated a human lung adenocarcinoma cell line (A549) with suspended particles collected during their epidemiological study (described in Section 4.1.7(a)(ii)). Exposures were categorized as SP1 (particles from wood smoke training), SP2 (from wood smoke training that also included electrical cords and mattresses in the fire), and TDEP (from train diesel exposure). Cells were treated with each at three non-cytotoxic concentrations over 2–4 weeks. SP1 was significantly associated with decreased telomere length only at 2 weeks. When pooling results from all three exposures, there was a significant decrease in telomere length within 4 weeks. [The Working Group concluded that the effect was in the same direction as that observed in the epidemiological study, but results were only statistically significant in the in vitro study, in which exposures were limited to the collected PM.]

(b) Alters cell proliferation, cell death, or nutrient supply

Only one study relevant to firefighting was found in the literature for the key characteristic "alters cell proliferation, cell death, or nutrient supply". The study assessed cell proliferation and viability in immortalized human cells in vitro. Kafkoutsou et al. (2022) treated human embryonic kidney cells (HEK-293) with three different class B AFFFs. The foams were collected from fire departments in the USA and contained either PFOA or an unspecified C6-fluorosurfactant. Cells were treated with each foam at seven concentrations (up to 10% in media), with the vehicle as the control. Cell viability and cell proliferation were assessed (the latter with the CellTiter 96 AQueous One solution MTS assay) after 72 hours of exposure. For all three foams, there were decreases in both cell viability and cell proliferation with increasing exposure concentration; concentrations of > 3% consistently showed significant decreases for all foams. The PFOA-containing foam exhibited cytotoxicity at the lowest concentrations. [The Working Group noted that this finding may be relevant to kidney toxicity.]

(c) Multiple characteristics identified by transcriptomics or other experimental approaches

See Table 4.13.

This section describes other studies relevant to cancer mechanisms: oncoproteins (Ford et al., 1992), an oncogenic growth factor (Min et al., 2020), and transcriptomics (Gainey et al., 2018).

Table 4.13 End-points relevant to multiple characteristics (other potential biomarkers and susceptibility factors)

End-point	Biosample, tissue, or cell type	Type of the exposure, location, setting, study design	No. of exposed and controls	Response (significance)[a]	Covariates controlled	Comments[b]	Reference
Proteins (2 growth factors and 7 oncoproteins)	Serum	Employment as firefighter USA (New York), New York City Fire Department, case–control	33 (selected from 226) firefighters, 16 controls (medical centre workers)	(+) ↑ TGFβ detection in firefighters (42%) compared with controls (0%)	Controls matched on age, sex, smoking status, race	Very small sample size; all men; method may have had low detection limit (no proteins detected except TGFβ)	Ford et al. (1992)
FGF-23, α-klotho, vitamin D	Serum	Employment as firefighter Republic of Korea, Sleep Panel Study (SLEPS), cross-sectional	450 (active firefighters including 81 day-only and 369 shift workers)	+ Shift work and job type associated with ↑ FGF-23 and α-klotho	Age, sex, BMI, LDL cholesterol (originally considered alcohol, smoking and exercise)	Strength: compared results across 5 job types and 5 shift types; 92% male participants; vitamin D was low among all firefighters	Min et al. (2020)

BMI, body mass index; FGF-23, fibroblast growth factor-23; LDL, low-density lipoprotein; TGFβ, transforming growth factor beta.

[a] +, statistically significant result(s) reported; no changes, no statistically significant results reported for any end-points of interest; (+) statistically significant result but study was of limited quality; ↑, increase.

[b] Factors to be considered for study quality included the methodology and design, reporting, and exposure assessment quality.

(i) Exposed humans

Ford et al. (1992) used immunoblotting to screen for nine serum oncoproteins and growth factors among a small sample of firefighters and controls (medical workers) from New York City, USA. Only transforming growth factor beta (TGFβ) was detected in any samples, and significantly more TGFβ was detected in firefighters (42%) than in controls (0%). [The Working Group noted that this marker is a regulator of cancer stemness and has been related to cancer risk and non-malignant respiratory disease in other studies (Bellomo et al., 2016; Saito et al., 2018).] The oncogene FGF-23, the tumour suppressor α-klotho, and vitamin D were measured in serum from firefighters (Min et al., 2020). [The Working Group noted that the focus of this study was circadian rhythm disruption among firefighters rather than other occupational exposures.]

(ii) Human cells in vitro

No data on human cells in vitro were available to the Working Group.

(iii) Experimental systems

Gainey et al. (2018) reported on a mouse model of fireground exposure, which demonstrated gene expression changes after exposure (also described in Section 4.1.5). The model was designed to test the acute impact of exposure during overhaul without SCBA protection. Male C57BL/6J mice were compared across three groups: control (never left animal facility), fireground exposure group (FG, stayed in the structure in a non-affected area), and overhaul group (OH, placed in area with overhaul). There were six mice in each group, and the experiment was repeated on three different days with new mice. RNA sequencing was performed on lung tissue collected 2 hours after overhaul. Of 16 261 genes detected, 1890 were significantly upregulated and 1962 were downregulated in the OH group compared with the FG group; this included 43 genes each with > 50% change in either direction. Differentially expressed genes were over-represented in 22 KEG (Kyoto Encyclopedia of Genes and Genomes) pathways, including chemical carcinogenesis, miRNAs in cancer, choline metabolism in cancer, and more.

4.2 Other relevant evidence

Studies reporting other evidence that may be relevant for carcinogenesis included assessment of hospital admissions from endocrine and metabolic disorders among firefighters, proteomics analyses after an exercise challenge, and a case series of allergic contact dermatitis in five firefighters (Ryu et al., 2021; Zhu et al., 2021; Patel & Nixon, 2022). However, the findings were deemed less informative and sporadic compared with the findings from other available studies.

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