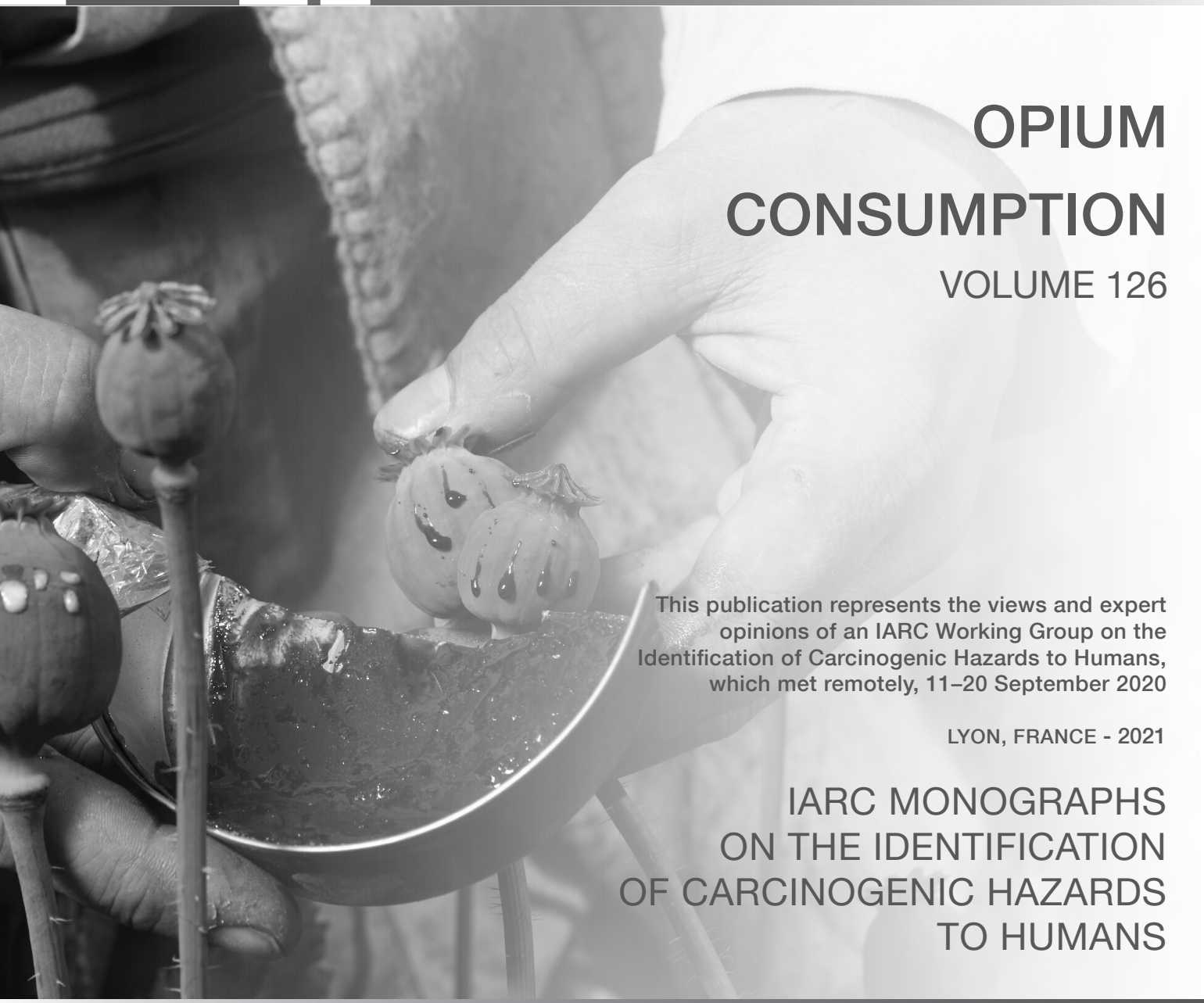
IARC MONOGRAPHS



OPIUM CONSUMPTION

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International Agency for Research on Cancer

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4. MECHANISTIC EVIDENCE

4.1 Absorption, distribution, metabolism, and excretion

This section describes the available evidence on the absorption, distribution, metabolism, and excretion of opium alkaloids after the consumption of opium by humans and experimental animals.

The biomedical effects of opium originate from the properties of either the major components of opium or their metabolic products. In the case of opium smokers, the products of pyrolysis should also be considered, although the structures of the products involved have not been clearly determined.

Direct studies characterizing rates of absorption after oral or inhalation exposure are sparse; however, evidence for absorption in humans and experimental animals is provided by the studies on intoxication and studies characterizing excretion described in Sections 4.1.1 and 4.1.2. Distribution of opium alkaloids to various tissues – and excretion including via the urine, gastrointestinal tract, and hair – has similarly been demonstrated both in humans and in rodents, as described below.

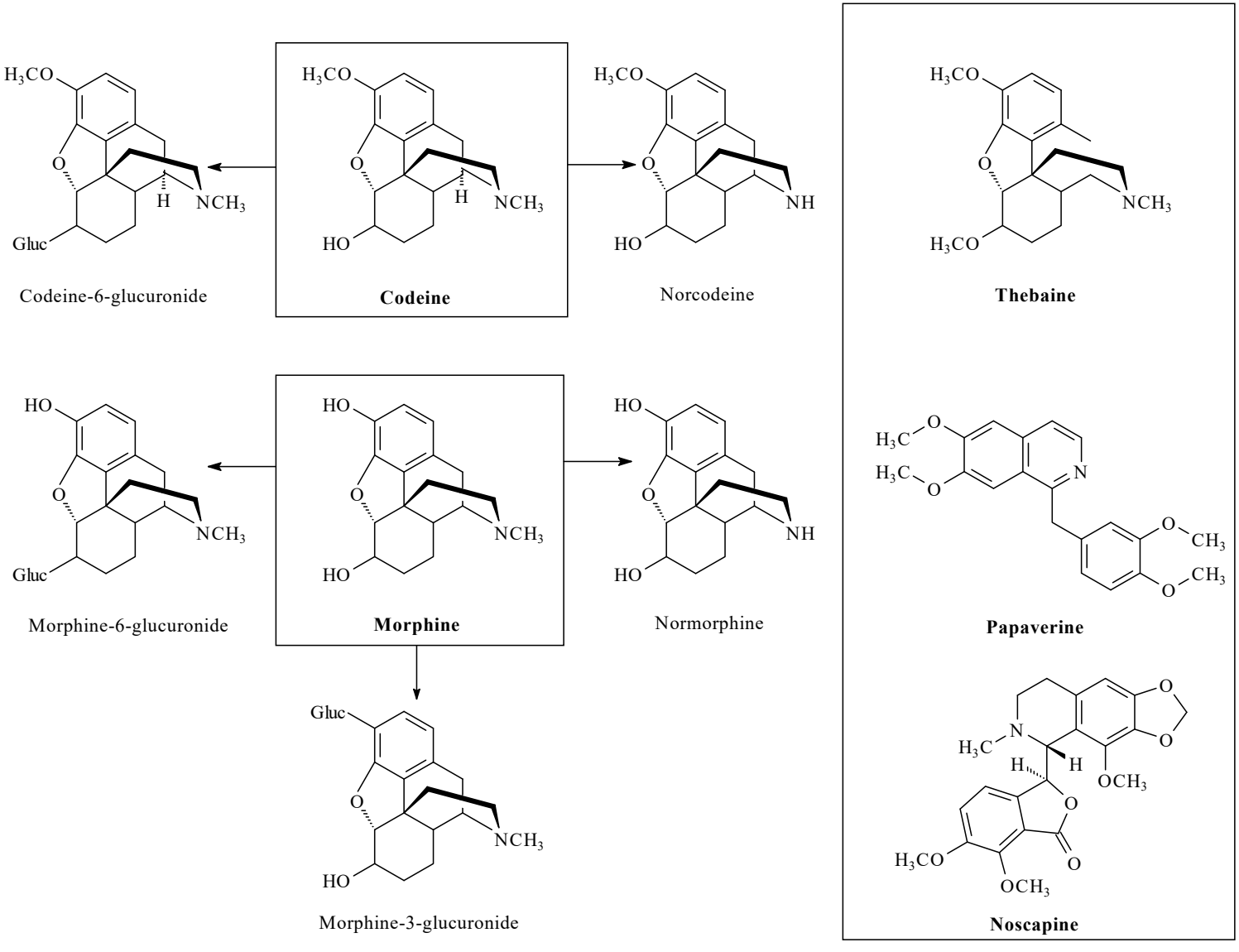
The metabolism of the major alkaloids in opium, such as morphine and codeine, has been well studied (Dinis-Oliveira, 2019). However, there are few reports on the pharmacokinetic properties of opium (mixtures of alkaloids and other components) in humans, except in the field of forensic toxicology. The primary site of morphine biotransformation is the free phenolic hydroxyl group at position 3 via which morphine is converted to inactive morphine-3-glucuronide (M3G; 57.3%); only a small percentage of morphine is converted via the alcohol group at position 6 to active morphine-6-glucuronide (M6G; 10.4%). Both conjugations are catalysed mainly by uridine 5′-diphospho-glucuronosyltransferase (UGT)2B7, but also by UGT1A1, UGT1A3, and UGT1A9 (Dinis-Oliveira, 2019). Codeine has an alcoholic hydroxyl available for glucuronidation only at position 6, leading to the formation of active/analgesic codeine-6-glucuronide, which is the major metabolite (80%). Of an oral dose of codeine, 0–15% is O-demethylated to morphine by the polymorphic enzyme cytochrome P450 2D6 (CYP2D6) and 10–15% is N-demethylated to norcodeine via CYP3A4 (Dinis-Oliveira, 2019). The chemical structures of the five major alkaloids in opium and their metabolites, described in this section, are shown in Fig. 4.1.

4.1.1 Humans

(a) Exposed humans

Morphine was detected in all hair samples collected from 30 opium smokers (all men who had been referred to a detoxification centre).

Fig. 4.1 Structures of the five major alkaloids in opium (shown in boxes) and their metabolites



Gluc, glucuronide.
Compiled by the Working Group.

The ages of the participants ranged from 21 to 51 years and their hair colour (natural or dyed) was reported to be black, blond, light brown, or white. Each participant had smoked 1–7.5 g of opium per day for 12–92 months. Gas chromatography-mass spectrometry analyses of extracts from the hair samples revealed a morphine concentration range of 0.26–10.31 ng/mg of hair. The higher the daily dose of opium, the higher the morphine concentration in hair. In addition, higher concentrations of morphine were detected in black hair than in hair of other colours (Sabzevari et al., 2004). In a hair sample obtained from a woman aged 50 years, in the Republic of Korea, who had cultivated opium poppies in her private garden and had ingested the liquid extracted from the poppies, thebaine (0.7 ng/mg), morphine (0.4 ng/mg), codeine (0.6 ng/mg), and norcodeine (below the limit of quantification) were detected (Lee et al., 2011). In a urine sample from a man who was an "opium eater", who had been hospitalized for treatment of cancer of the oesophagus, morphine (0.64 µg/mL) was detected at nearly twice the concentration of codeine (0.37 µg/mL), while normorphine and norcodeine were detected in equal amounts (about 0.15 µg/mL). The patient had ingested approximately 1 g per day of a dark, resinous material that he identified as *sukhteh* [opium dross] from his opium pipe. After the urine sample had been treated with β-glucuronidase to hydrolyse the conjugated metabolites, the concentrations of the four compounds described above increased by more than 10 times. There were no traces of thebaine, papaverine, or oripavine after the sample had been treated (Cone et al., 1982). In the case of a sudden fatality (a man aged 32 years) involving opium consumption in a legal poppy field in Spain, thebaine (0.10, 7.12, 0.23, and 14.80 mg/L), morphine (0.13, 4.50, 0.13, and 6.60 mg/L), and codeine (0.48, 0.88, 0.17, and 1.50 mg/L) were detected in the man's peripheral blood, urine, vitreous humour, and gastric contents, respectively. Other toxicological findings included the presence of metabolites of cocaine and cannabis (Martínez et al., 2016).

Reticuline is a minor alkaloid in opium (0.001–0.3%, w/w) and it is a precursor of the principal opium alkaloids thebaine, morphine, and papaverine. Analyses from a forensic laboratory showed that 291 urine samples from opium users (their intake route was uncertain) contained reticuline and morphine. The percentage concentration ratios of reticuline : morphine (2–12) in these urine samples were higher than those in opium (0.01–3). As well as being a constituent of opium, reticuline in the urine of opium users may also result from the metabolic demethylation of the three other benzyltetrahydroisoquinoline opium alkaloids: codamine, laudanosine, and laudanine (Al-Amri et al., 2004). Extracts of 100 urine samples obtained from forensic case studies, which had previously yielded positive results in an immunoassay for opiates, were examined by gas chromatography-mass spectrometry. Neopine was detected in urine samples obtained from both opium users and pharmaceutical codeine users but could not be detected in urine samples obtained from confirmed heroin users. Neopine, a minor opium alkaloid, has been identified as a metabolite of codeine in humans, and may be a marker of opium use or pharmaceutical codeine and heroin use (Al-Amri et al., 2005).

Urinary levels of metabolites of several toxicants and carcinogens were higher among exclusive long-term users of opium and dual users of opium and cigarettes than non-users. Urine from opium users contained high concentrations of several toxicant and carcinogen metabolites, and dual users of opiates and cigarettes had higher concentrations of all biomarkers than people who used cigarettes or opium exclusively. Opium consumption contributed substantially to the levels of many of these metabolites, particularly those of polycyclic aromatic hydrocarbons and some volatile organic compounds, namely metabolites of acrylamide, 1,3-butadiene, and

dimethylformamide. Among the toxicant and carcinogen biomarkers present at high concentrations in opium users, most were present at similar concentrations regardless of route of use (ingestion or smoking), except for a few that were associated with smoking opium (Etemadi et al., 2020).

(b) Studies on volunteers

The maxima in the hourly urinary excretion patterns of morphine occurred 2–4 hours after single doses of a medicinal opium mixture containing 2.5 mg of morphine and smaller amounts of codeine, together with a kaolin solution, were administered orally to six volunteers. The urinary excretion of morphine appeared to be a more gradual process than that observed after the consumption of medicinal morphine hydrochloride (equivalent to 1.5 mg of morphine base). The morphine concentrations in urine were generally below 1.0 μg/mL. A significant amount of codeine was also detected in each urine sample. The codeine:morphine ratio ranged from 0.1 to 0.7. In total, the amount of morphine (free and conjugated) excreted during an 8-hour period after consumption was found to be in the range of 6–17%. Although a single dose of opium contained more morphine than a single dose of medicinal morphine, the total urinary excretion of morphine after the consumption of opium was about four times less than in the case of medicinal morphine (Yong & Lik, 1977).

Urinary excretion of both morphine and codeine reached their maxima 2–6 hours after ingestion of a single dose of either tablets or a solution of Brown Mixture (BM), which is a legal prescription drug in Taiwan, China, and contains opium powder, opium tincture, or camphorated opium tincture. Single oral doses of one, two, four, or six BM tablets (each tablet contained 281.11 μg of morphine and 32.41 μg of codeine) were administered to four volunteers. Single oral doses of 5, 10, 15, or 20 mL of BM solution (containing opium tincture, with morphine and codeine at concentrations of 134.91 μg/mL and 46.85 μg/mL, respectively) were administered to the same four volunteers, respectively, 2 weeks after completion of the first experiment. In addition, multiple oral doses (three times per day for 2 days) of one, two, or four BM tablets were administered to three additional volunteers. Multiple oral doses of 5, 10, and 15 mL of BM solution were administered to the same three volunteers, respectively, 2 weeks after the completion of the first experiment. Urine was collected at 0, 2, 4, 6, 8, 10, 12, 14, and 16 hours, and then every 4 or 8 hours until both codeine and morphine became undetectable (< 0.05 μg/mL). Morphine concentrations found in urine specimens collected from the volunteers were always < 4 μg/mL. Depending on the dose administered, morphine became undetectable 24–42 hours after a single dose, while codeine disappeared more quickly (8–18 hours). Morphine:codeine ratios observed in urine specimens with morphine concentrations of < 300 μg/mL were: (i) less than 3.0 for volunteers ingesting BM solution and (ii) greater than 3.0 (mostly > 5.0) for volunteers ingesting BM tablets (Liu et al., 2006).

Plasma morphine concentrations differed significantly across dosing groups (6.66, 13.3, and 20 mg of morphine equivalents, twice per day) in a study of 45 opium-dependent Thai participants who were allocated to one of three different dosing groups depending on their self-reported prior opium use. On day 5 of the dosing period, an interdosing interval study was conducted in which blood samples were taken from participants, and their withdrawal scores, heart rates, and blood pressure were assessed at 0, 1, 3, and 8 hours. Plasma morphine concentrations changed significantly across the interdosing interval for all three doses ($P = 0.0001$), increasing to a maximum 1 hour after administration and then decreasing rapidly to a minimum 8 hours after ingestion. The mean ratios of the morphine glucuronides M3G and M6G were: M3G:M6G,

7.7; M3G:morphine, 35.6; and M6G:morphine, 4.9 (Somogyi et al., 2008).

4.1.2 Experimental systems

Thebaine, codeine, norcodeine, and morphine were detected in the dark grey hair of three male lean Zucker rats given an opium suspension, prepared by shaking opium in saline, at a dose of 100 mg/kg, once per day for 2 weeks. Before dosing began, areas of dark grey and white hair were separately shaved. These areas were shaved again and hair collected 2 weeks after administration of the final doses. The mean concentrations of thebaine, codeine, norcodeine, and morphine in the dark grey hair were 3.2, 2.6, 0.6, and 1.2 ng/mg, respectively. Normorphine was also detected in the dark grey hair but was below the limit of quantification. No opiates were detected in the white hair (Lee et al., 2011).

There was only one report that described compounds in vapour derived from the volatilization of opium, and the urinary excretion of these compounds after inhalation of volatilized opium by experimental animals (Kikura-Hanajiri et al., 2003). In three male Wistar rats exposed to opium by vapour inhalation for 20 minutes, the following compounds were detected in urine collected over a 72-hour period: M3G (5.45–14.38 μg), morphine (2.27–4.65 μg), meconin (4.60–5.06 μg), codeine (0.54–1.85 μg), noscapine (0.34–0.40 μg), and papaverine (0.01–0.04 μg). Only a trace level of thebaine was observed.

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether opium consumption is genotoxic; alters cell proliferation, cell death, or nutrient supply; induces chronic inflammation; is immunosuppressive; or induces oxidative stress. Insufficient data were available for the evaluation of other key characteristics of carcinogens.

4.2.1 Is genotoxic

Table 4.1 and Table 4.2 summarize the identified studies relevant to whether opium is genotoxic.

(a) Exposed humans

Abedi-Ardekani and co-workers characterized the mutation pattern of the oncosuppressor gene *TP53* in tumour biopsies collected from 119 patients with oesophageal squamous cell carcinoma who were enrolled in a case–control study in Golestan, a north-eastern province of the Islamic Republic of Iran, an area where the incidence of oesophageal squamous cell carcinoma is one of the highest in the world (Abedi-Ardekani et al., 2011). Only 15 (12.6%) and 21 (17.6%) of the participants reported using opium or both tobacco and opium, respectively. The molecular analysis of the mutational spectrum revealed the highest rate of *TP53* mutation (89.9%) ever reported to date, anywhere and in any cancer (107/119 cases, 15/15 opium users, and 17/19 opium and tobacco users had at least one mutation). Direct sequencing of *TP53*, exons 2 through 11, showed a heterogeneous pattern of mutations likely due to the additive action of several environmental carcinogens (Abedi-Ardekani et al., 2011).

Specifically, GC→AT transitions, not located at cytosine-phosphate-guanine (CpG) sites, were the most common mutations (25%) followed by GC→TA transversions (16.7%). GC→AT transitions can be the result of exposure to several mutagens, such as alkylating agents, nitrosoamines, and nitric oxide (NO), therefore preventing the assignment of this mutation to a single category of mutagens. However, GC→TA transversions are the most common mutations induced by polycyclic aromatic hydrocarbons, which

Table 4.1 Genetic and related effects of opium in exposed humans

End-point	Tissue, cell type	Location, setting, study design	Exposure level and number of exposed and controls	Response[a]	Covariates controlled	Comments	Reference
Gene mutation, *TP53* exons 2 through 11 (direct sequencing)	Biopsies of 119 oesophageal squamous cell carcinomas	Golestan, Iran (Islamic Republic of), case–control study	15 opium users 21 opium and tobacco users, 67 neither Lower numbers for specific mutations	(–)	Age, sex, ethnicity, tobacco consumption (ever/never), tea temperature, and residence (urban/rural)	Mutation patterns differed with temperature of tea consumed, but not with opium use. Small number of opium users, especially for specific mutations. Opium exposure was poorly defined and specified.	Abedi-Ardekani et al. (2011)

[a] (–), negative in a study of limited quality.

Table 4.2 Genetic and related effects of opium in human cells in vitro and in experimental systems

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Human cells						
Sister-chromatid exchange	Human PBMCs	+	+ (0.5% S9 mix)	Opium pyrolysates or *sukhteh* [opium dross], 30 µg/mL	Small sample size (three healthy donors). Dose–response relationships.	Perry et al. (1983)
Non-human mammalian cells						
Sister-chromatid exchange	Chinese hamster ovary cells	+	+ (0.5% S9 mix)	Opium pyrolysates or *sukhteh* [opium dross] (approximate dose range, 5–100 µg/mL) LEC, 5 µg/mL	Dose–response relationships.	Perry et al. (1983)
Non-mammalian systems						
Base substitution (TA100) and frameshift mutations (TA98) (Ames test)	*Salmonella typhimurium* TA100 and TA98	NR	± (TA98)	Crude opium	Crude opium, six samples.	Malaveille et al. (1982)
		NR	– (TA100)			
		±	+	*Sukhteh* [opium dross]	*Sukhteh*, 21 samples; TA98 > TA100.	
		+	+	Opium pyrolysates	Opium pyrolysates from four countries all + in both strains with and without activation; concentration-dependent relationships with activation; TA98 > TA100.	
Frameshift mutations (Ames test)	*Salmonella typhimurium* TA1538	NT	+	*Sukhteh* [opium dross]		Malaveille et al. (1982)
		NT	+	Opium pyrolysates		
Base substitution (TA100) and frameshift mutations (TA98) (Ames test)	*Salmonella typhimurium* TA100 and TA98	–	+	*Sukhteh* [opium dross]: LEC, 2080 µg/plate (TA100) LEC, 800 µg/plate (TA98)	Concentration-dependent relationship for mutagenic effect of *sukhteh* in both strains; T98 > TA100	Hewer et al. (1978)
		–	–	*Shireh* [a minimally refined opium product]: HIC, 6250 µg/plate	Non-mutagenicity of *shireh* may be due to preassay processing.	
		–	± (TA98)	Crude opium	Questionable purity of some crude opium samples.	
		–	– (TA100)			

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; PBMC, peripheral blood mononuclear cell.

[a] +, positive; –, negative; ±, equivocal (variable response in several experiments within an adequate study).

are human carcinogens produced by pyrolysis, including during opium smoking. [The Working Group noted that the number of opium-only users was small, especially for analyses of specific *TP53* mutations, and opium exposure was poorly defined and specified.]

(b) Human and other mammalian cells in vitro

In vitro cell culture studies show that *sukhteh* [opium dross] and opium pyrolysates induce sister-chromatid exchanges in both human peripheral blood mononuclear cells and Chinese hamster ovary cells. A clear dose–response relationship was observed in both cell types with or without metabolic activation, indicating the presence of direct clastogenic agents in opium pyrolysates (Perry et al., 1983).

(c) Non-mammalian experimental systems

Assays for mutagenicity or genotoxicity in bacteria using *Salmonella typhimurium* strains TA98 and TA100 have been performed for various opium products. In an early study, six samples each of *sukhteh* [opium dross] and *shireh* [a minimally refined opium product], and three samples of crude opium, were collected in villages in the north-east of the Islamic Republic of Iran and in Transkei, South Africa, and three samples of crude opium from other countries were obtained from the French Ministry of Health (Hewer et al., 1978). A concentration-dependent increase in mutagenesis was observed for *sukhteh* in both strains with rat liver microsomal activation. At each tested concentration, the mutagenicity induced by *sukhteh* was higher in the TA98 than in the TA100 strain (Hewer et al., 1978). Although it sometimes contains *sukhteh*, *shireh* exhibited little mutagenic activity in either strain, possibly due to processing before the assay was conducted (Hewer et al., 1978). The crude opium samples showed no mutagenic activity, with the exception of three of the village samples, which may have been contaminated with *sukhteh*, which is often mixed with crude opium (Hewer et al., 1978). These early results were confirmed by a larger study, which tested 21 samples of *sukhteh* [opium dross] and 6 of raw opium, as well as opium pyrolysates from four different countries (Malaveille et al., 1982). In addition, *sukhteh* and opium pyrolysates induced frameshift mutations in *Salmonella typhimurium* strains TA98 and TA1538 (Malaveille et al., 1982).

4.2.2 Alters cell proliferation, cell death, or nutrient supply

Table 4.3 summarizes the identified studies relevant to whether opium alters cell proliferation, cell death, or nutrient supply.

(a) Exposed humans

Compared with those from non-tobacco smokers, smears of buccal mucosa and mouth floor samples obtained from smokers and opium-addicted participants were characterized by an increase in both nuclear diameter and nuclear:cytoplasmic ratio, as well as by a decrease in cellular size. The smears were collected from a cohort of 300 men (100 controls, 100 tobacco smokers, and 100 opium addicts). (Hashemipour et al., 2013). [The Working Group noted that the cigarette-smoking status of the opium user group was not reported and as such there was no attempt to distinguish the effects of cigarette smoking. Cytomorphometry may not represent cell proliferation.]

The effect of opium consumption on argyrophilic nucleolar organizer region (AgNOR) changes in buccal mucosa cells was evaluated in a cohort of men and women that included non-smokers, tobacco smokers, and opium addicts. The opium addicts included tobacco smokers, with the average cigarette consumption per day being similar in the two groups (18.7 in opium addicts vs 18.4 in tobacco smokers). Exfoliative cytological analysis showed a higher AgNOR count in smokers than in controls and

Table 4.3 End-points relevant to cell proliferation and death in exposed humans

End-point	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)[a]	Covariates controlled	Comments	Reference
Cytomorphometry	Smears of buccal mucosa and mouth floor	Kerman City, Iran (Islamic Republic of), cross-sectional	100 opium addicts (≥ 4 g/day during ≥ 3 of the last 6 yr; selected by DSM-IV-TR criteria for addiction), 100 tobacco smokers, 100 non-smokers; ≥ 4 g opium/day	+ Different rate of keratinization and significant[b] differences in cellular size of epithelial cells in opium addicts vs non-smokers	Tobacco; users of alcohol and drugs affecting oral epithelium excluded	Questionnaire, no details of questions or whether administered or self-completed. Well-defined opium use except that type of opium is not presented; had to be recent opium use and ≥ 4 g/day; grams per day and duration of addiction collected; also collected tobacco smoking per day and duration; alcohol users excluded.	Hashemipour et al. (2013)
AgNOR count	Smears of buccal mucosa	Tehran, Iran (Islamic Republic of), cross-sectional	25 opium addicts (exposure level NR; average duration 12.8 yr), 25 tobacco smokers (average cigarette consumption/day similar, 18.7 in opium addicts vs 18.4 in tobacco smokers), 25 non-smokers	+ ($P < 0.0001$) Opium addicts (9.21 ± 2.95) > tobacco smokers (5.68 ± 2.17) > non-smokers (4.3.5 ± 1.62)	Opium addicts include tobacco smokers, then opium addicts are compared with tobacco smokers	Assessment method not given; very little information on exposure, which is poorly defined and characterized; many of the opium addicts also smoked.	Kadivar & Attar (2008)

AgNOR, argyrophilic nucleolar organizer region; DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision; NR, not reported; vs, versus; yr, year.

[a] +, positive

[b] Statistical significance was defined as $P < 0.05$ (Mann–Whitney test and Student's *t*-test).

in opium addicts than in smokers (Kadivar & Attar, 2008). [The Working Group noted that the AgNOR end-point lacks specificity for reflecting cell proliferation.]

(b) Human cells in vitro and other experimental systems

No data were available to the Working Group. [The Working Group noted that opium has been demonstrated to have pro-apoptotic activity in human cells in vitro (Khaleghi et al., 2016) and in rodents in vivo (Asiabanha et al., 2011; Asadikaram et al., 2013a). Opium-induced apoptosis and necrosis has also been reported in Jurkat cells (an immortalized line of human T-lymphocyte cells) (Igder et al., 2013); see Section 4.2.3.]

4.2.3 Induces chronic inflammation or is immunosuppressive

See Table 4.4.

(a) Exposed humans

(i) Cytokines

Most of the available studies in humans have compared cytokine levels in opium users and non-users.

In a study of patients with documented three-vessel coronary artery disease, 15 cigarette-smoking men with opium addiction were compared with 15 cigarette-smoking non-addicted men. Levels of interleukin (IL) 1R antagonist, an acute-phase inflammation marker, were significantly higher in the addicted group, while levels of IL6 were similar between the two groups. All patients performed a treadmill test, and levels of cytokines were measured before, immediately after, and 4 hours after the test (Saadat et al., 2012). [The Working Group noted that the exposure was defined as "patients with only opium addiction (raw opium inhalation)" and that no details were given about how the opium history was obtained. The Working Group also noted that no details regarding the levels of cigarette smoking across groups were given.]

In a study of 30 male opium addicts and matched controls, plasma levels of IL4 and interferon γ (IFNγ) were significantly lower, and IL6 and transforming growth factor β (TGFβ) were significantly higher, in opium-addicted participants than in controls. Individuals who smoked tobacco or consumed other substances (any medication, other components of opium such as morphine, heroin, and drugs for the treatment of heroin withdrawal such as methadone) were excluded (Nabati et al., 2013). The study also included in vitro evaluation of lymphocytes from both groups with and without opium treatment ("culturing with opium"), as described in Section 4.2.3b. [The Working Group noted that no details were given about how opium history was obtained, and that the publication contains few details about the characteristics of addicts and non-addicts.]

In a study by Ayatollahi-Mousavi et al. (2016) that examined the associations between cytokine concentrations and opium addiction in opium addicts with or without fungal infection, 72 individuals in four groups of 18 individuals each (opium addicts/non-opium-addicts, with/without fungal infection) were assessed. Two-way analysis of variance (ANOVA) showed that levels of IL17, TGFβ, and IFNγ in blood plasma differed significantly between opium addicts and non-addicts, whereas levels of IL4 and IL6 did not. [The Working Group noted that the analyses did not sufficiently account for fungal infection.] After excluding the 36 individuals with fungal infection, IL17 levels in opium addicts were significantly higher than those in non-addicts. The differences reported for IFNγ, TGFβ, IL4, and IL6 levels were not statistically significant based on the Working Group's analysis of opium users and non-users without fungal infection. [The Working Group noted that

Table 4.4 Effects of opium use on immune function and inflammation in exposed humans

End-point	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
Cytokines (IL1 receptor antagonist and IL6 levels) ESR	Plasma	Tehran, Iran (Islamic Republic of), patients with three-vessel coronary artery disease (all men), cross-sectional	Exposure level, NR 15 cigarette smokers (mean age 54.7 ± 1.7 yr) with opium addiction were compared with 15 non-addicted sex-, age-, and cigarette-smoking-matched patients	Higher IL1Ra plasma levels in the addicted patients compared with the non-addicted patients (before, immediately after, and 4 h after treadmill test in all patients) ($P = 0.015$) No significant changes in IL6 plasma levels and ESR	NR	Poorly defined and poorly characterized exposure; exposure definition was "patients with only opium addiction (raw opium inhalation)". No details were given about how the opium history was obtained. All patients were current tobacco smokers; however, no details on levels of tobacco smoking across groups were provided. IL1Ra and IL6 levels were measured in conjunction with treadmill test; ESR results were based on single measurement per patient.	Saadat et al. (2012)

Table 4.4 Effects of opium use on immune function and inflammation in exposed humans (continued)

End-point	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
Cytokines (IL4, IFNγ, IL6, and TGFβ levels)	Plasma	Kerman, Iran (Islamic Republic of), opium addicts and non-addicted controls (all men), cross-sectional	30 opium-addicted individuals (aged 19–56 yr; smoking > 0.5 g/day for ≥ 1 yr) and 30 non-addicted age-, residence-, and BMI-matched controls	Lower levels of IL4 in addicts (15.11 ± 0.5561 pg/mL) compared with controls (20.57 ± 0.9420 pg/mL) ($P < 0.0001$) Lower levels of IFNγ in addicts (13.43 ± 0.5673 pg/mL) compared with controls (44.91 ± 3.995 pg/mL) ($P < 0.0001$) Higher levels of IL6 in addicts (367.2 ± 14.42 pg/mL) compared with controls (238.2 ± 8.596 pg/mL) ($P < 0.0001$) Higher levels of TGFβ in addicts (1657 ± 73.36 pg/mL) compared with controls (1028 ± 63.74 pg/mL) ($P < 0.0001$)	NR	Well-defined but poorly characterized exposure; minimum amount of opium smoked per day to enter the study, but type not presented; years of exposure not mentioned and amount of opium not presented; individuals excluded if tobacco smokers or consumers of opiates or other medications; control group non-opium users and non-tobacco smokers; source of addicts and control individuals NR. No details were given about how the opium history was obtained.	Nabati et al. (2013)

Table 4.4 Effects of opium use on immune function and inflammation in exposed humans (continued)

End-point	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
Cytokines (IL4, IL6, IL17, IFNγ, and TGFβ levels)	Plasma	Kerman, Iran (Islamic Republic of), male hospital attendees, cross-sectional	Addicted to opium (smoked and/or ingested for ≥ 3 yr) without FI (n = 18); non-addicted controls without FI (n = 18); mean age, 33.43 ± 5.22 yr	Higher levels of IL17 in addicts (159.10 ± 47.45 pg/mL) compared with controls (121.17 ± 26.62 pg/mL) (significance, NR) Lower levels of IFNγ in addicts (75.56 ± 37.23 pg/mL) compared with controls (88.74 ± 20.11 pg/mL) (significance, NR) Higher levels of TGFβ in addicts (731.05 ± 259.80 pg/mL) compared with controls (683.88 ± 94.76 pg/mL) (significance, NR) No significant changes in IL4 and IL6 plasma levels	NR	Poorly characterized exposure; opium exposure defined as addict or not, with no details on the intensity and type of opium. Questionnaire about smoking and narcotic drug use, but no details given. Exclusion criteria: being female, aged < 18 yr or > 60 yr, and taking immunosuppressive drugs Did not include statistical analysis comparing opium users and non-users with and without FI; the Working Group's analysis (t-test) showed that only the difference in IL17 was significant.	Ayatollahi-Mousavi et al. (2016)

Table 4.4 Effects of opium use on immune function and inflammation in exposed humans (continued)

End-point	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
Cytokines (TNFα)	Plasma	Mazandaran, Iran (Islamic Republic of), opium users during an MTT programme and healthy non-smoking controls (all men), cross-sectional study (baseline data from intervention study)	20 tobacco-smoking opium addicts (> 1 g/day for ≥ 1 yr). 40 controls (20 tobacco smokers/20 non-smokers)	Higher TNFα in patients before methadone therapy (199.96 ± 69.14 pg/mL) compared with the tobacco smoker (141.23 ± 96.2 pg/mL) or non-smoker (40.22 ± 25.8 pg/mL) comparison groups ($P < 0.05$) TNFα levels decreased significantly during methadone treatment	NR	Data collected at clinical interview; opium use validated by opioid detected in urine at baseline. Well-defined, validated exposure, unclear if collected intensity and duration except to confirm minimum exposure; type of opium and method of exposure not presented; comparison group clearly unexposed to opium, but source of subjects not described. Other substance users excluded by urine tests. Levels of smoking slightly higher in opium users than in tobacco-smoking controls (1.3 vs 1.1 pack-years).	Salarian et al. (2018)

Table 4.4 Effects of opium use on immune function and inflammation in exposed humans (continued)

End-point	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
Cytokines (IL4, IL10, IL17, and IFNγ levels) hs-CRP	Serum/ plasma	Arak, Iran (Islamic Republic of), opium addicts from a detoxification centre and healthy individuals with no history of drug abuse as the control group (all men), cross-sectional	44 opium addicts from a detox centre (aged 20–40 yr; mean, 31 yr) who smoked opium, > 2 g/day (range, 2000–3000 mg) for ≥ 1 yr; 44 age-, sex-, SES-, and tobacco-smoking status-matched controls	Higher levels of IL10 (95.48 ± 13.05 pg/mL) in opium users compared with controls (66.28 ± 2.62 pg/mL) ($P < 0.026$) Higher levels of IL17 (19.23 ± 0.64 pg/mL) in opium users compared with controls (16.99 ± 0.15 pg/mL) ($P < 0.001$) Higher levels of IFNγ (521.15 ± 33.08 pg/mL) in opium users compared with controls (399.44 ± 19.30 pg/mL) ($P < 0.002$) Higher levels of hs-CRP (8.93 ± 1.93 mg/mL) in opium users compared with controls (0.72 ± 0.09 mg/mL) ($P < 0.0001$) No significant changes in IL4 plasma levels	NR	No details were given about how the opium history was obtained, probably by questionnaire. Well-defined exposure consumption of opium (> 2 g/day for ≥ 1 year), which was not further characterized; confirmed by urine tests; smoked as *teriak*; years of exposure not collected. Polydrug abusers and alcohol users excluded; tobacco smokers included. Controls recruited by public announcement.	Ghazavi et al. (2013a, b)

Table 4.4 Effects of opium use on immune function and inflammation in exposed humans (continued)

End-point	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
hs-CRP	Plasma	Isfahan, (Islamic Republic of), opium addicts from a rehabilitation centre, and non-opium-addicted current smokers as controls (all men), cross-sectional	360 opium addicts (smoking opium for 5 mo to 5 yr), route of administration (orally, *vafoor*, and *sikh-sang*), all cigarette smokers; 360 non-opium addicts but current smokers, age- and cigarette/day-matched controls The mean number of smoked cigarettes/day was 15 ± 2 and 16 ± 3 in the opium-addicted and control groups, respectively The mean age was 38 ± 5 yr in the cigarette-smoking control group and 41 ± 3 yr in the opium-addicted group	Higher levels of CRP (4.11 ± 0.7 mg/dL) in opium users compared with controls (3.54 ± 0.3 mg/dL) ($P < 0.029$)	NR	Opium exposure defined as "opium addict" and assessed for oral and two inhalation routes; duration of addiction measured but not intensity. Questionnaire about smoking and narcotic drug use, no details. Study on cardiovascular risk factors.	Asgary et al. (2008)
CD4+ T-cell count		Tehran, (Islamic Republic of), HIV infected referred to an HIV/AIDS reference laboratory, Imam Khomeini hospital, case series	5 "patients who used opium" among 99 HIV-infected patients; exposure level, NR	Lower CD4+ T-cell count (245.68 ± 21.8 cells/mm^3) in opium users compared with controls (367.40 ± 40.7 cells/mm^3) ($P < 0.008$)		Study on HIV-infected patients, only 5/99 used opium; exposure poorly defined and characterized with type of opium, duration, and exposure method not presented. Common route was injection, but various drugs included. Study on clinical and laboratory profiles of patients with HIV.	Mohammadi et al. (2016)

AIDS, acquired immunodeficiency syndrome; BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FI, fungal infection; h, hour; HIV, human immunodeficiency virus; hs, high-sensitivity; IFN, interferon; IL, interleukin; IL1Ra, interleukin-1 receptor antagonist; mo, month; MTT, methadone maintenance treatment; NR, not reported; SES, socioeconomic status; TGF, transforming growth factor; TNF, tumour necrosis factor; vs, versus; yr, year.

the lack of adjustment for cigarette smoking was also a major limitation.]

A study of 60 individuals (20 with opium addiction, 20 cigarette-smoking controls, and 20 non-smoking controls) included follow-up of opium users during a methadone maintenance treatment programme designed to help them quit opium use. During transition from opium to methadone, blood and urine samples of the participants were periodically tested for opium use to ensure quitting. Opium users had higher plasma levels of tumour necrosis factor α (TNFα; an inflammation mediator) than both cigarette-smoking and non-smoking controls. During the methadone maintenance treatment programme, levels of TNFα dropped significantly until day 14 (when the study ended). Data were collected at clinical interview including diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) diagnosis, and opium use was validated by blood and urine test. Users had consumed opium for an average of 9.6 years and used on average 2.9 g per day; 60% smoked opium (Salarian et al., 2018). [The Working Group noted that the characteristics of users and controls were fully described.]

Two papers have been published that concern a single study of 44 opium addicts who voluntarily enrolled for detoxification and 44 matched controls recruited by "public announcement" (Ghazavi et al., 2013a, b). Serum concentrations of IFNγ, IL10, and IL17 in opium addicts were all significantly higher than in controls, but concentrations of IL4 were similar between the two groups (Ghazavi et al., 2013a). Opium addicts in this study also had increased levels of C-reactive protein (CRP), C3 and C4 complements, and immunoglobulin A, but not immunoglobulin M (Ghazavi et al., 2013b). [The Working Group noted that these two papers contain little information about the characteristics of addicts and non-addicts, including tobacco use, although controls were described as being matched to cases for cigarette smoking.]

(ii) General inflammation markers

In addition to the above study by Ghazavi et al. (2013a), which showed higher CRP levels in opium addicts compared with healthy controls with no lifetime history of substance abuse, two other studies have investigated general inflammation responses in opium users. In one study, described above (Saadat et al., 2012), the erythrocyte sedimentation rate was found to not differ significantly between opium addicts and non-addicts. In another study of "cardiovascular risk factors" in 360 opium-addicted individuals (who were also cigarette smokers) and 360 current cigarette smokers with no opium addiction, the concentrations of CRP were reported to be higher in individuals with opium addiction (Asgary et al., 2008).

In a study of 99 HIV-positive individuals, the five opium users had significantly higher CD4 counts than other groups of HIV-infected individuals. No additional data about opium use, the method of obtaining history of opium use, and other confounders were given (Mohammadi et al., 2016).

(b) Human cells in vitro

In a study of in vitro production of IFNγ and IL10 after antigenic stimulation of whole blood cells, 10 opium addicts were compared with 10 heroin addicts and 10 healthy controls (all groups consisted of men aged 20–40 years). Compared with healthy controls, levels of IFNγ decreased and IL10 increased in the whole blood cells from both opium and heroin addicts after antigenic stimulation. The changes in IFNγ and IL10 in the cells from opium addicts were less significant than those from heroin addicts. All individuals had negative test results for HIV and hepatitis B surface antigen. The addicts had used opium for an average of 8.7 years and were enrolled in a detoxification programme (Azarang

et al., 2007). [The Working Group noted that the study provided no details about how the opium history was obtained and how the controls were selected, nor did it mention cigarette smoking in any group.]

Lymphocytes from 30 male opium addicts and their matched controls (study described in Section 4.2.3a; Nabati et al., 2013) were studied in vitro with opium treatment (2.86×10^{-5} g/mL for 48 hours; "culturing with opium") and without. The plasma concentrations of IL4 and IFNγ in opium-addicted participants were significantly lower than those in the control group, while the concentrations of IL6 and TGFβ were significantly higher. The concentrations of all four cytokines in the in vitro supernatants of lymphocytes from opium-addicted participants were significantly lower than those from the control group. In the in vitro supernatants of lymphocytes from opium-addicted participants, concentrations of IL4, IL6, and TGFβ, but not IFNγ, decreased significantly in response to opium treatment. Culturing with opium increased IFNγ secretion by lymphocytes from the control group but did not affect the levels of other cytokines (Nabati et al., 2013).

Exposure of Jurkat cells to different concentrations of opium increased the secretion of IL6, decreased the secretion of TGFβ, and initially decreased IFNγ but later increased its secretion. These effects varied according to the opium dose and duration of treatment (Asadikaram et al., 2015).

The effects of opium on the induction of apoptosis and necrosis in Jurkat cells have been studied in two publications. In the first, the cells were treated with different concentrations of opium (2.86×10^{-3} or 2.86×10^{-11} g/mL) and compared with untreated cells (controls) after 6, 24, and 72 hours (Igder et al., 2013). Some of the opium-treated cells showed increased apoptosis after 6 hours, and there seemed to be a dose–response association at 24 hours. [The Working Group noted that the 72-hour results were inconsistent with a dose–response association.] There was evidence of increased necrosis with some of the opium concentrations at 24 and 72 hours. [The Working Group noted that the necrosis was not dose-dependent, nor was it consistent across different times since exposure.] The second study showed 50% of cells had apoptotic features (messenger RNA (mRNA) for pro-apoptotic and anti-apoptotic molecules) among cells treated with different concentrations of opium after 48 hours (Arababadi & Asadikaram, 2016). [Again, the Working Group noted there was no clear correlation with the opium concentration. In addition, the results for mRNA patterns and anti-apoptotic molecules did not include adjustment for multiple testing.]

(c) Experimental systems

In rats given two daily doses of opium of 30–150 mg/kg bw administered intraperitoneally at 08:00 and 20:00 for 9 consecutive days, there was a slight decrease in levels of TGFβ in males, but a significant increase in females compared with controls (Asadikaram et al., 2010). Increased neutrophil counts and decreased lymphocyte counts in peripheral blood of male and female rats were also observed compared with controls (Asadikaram et al., 2013b).

Plasma levels of IFNγ were increased and of IL4 were decreased before and after surgical stress in opium-addicted rats. Differences in IL10 and TNFα levels were not statistically significant (Lashkarizadeh et al., 2016).

Intraepithelial lymphocytes from the ilea of guinea-pigs that had been treated with 1 mL of deodorized opium tincture (orally) 2 hours before cell collection showed deficient natural killer cytotoxicity and antibody-dependent cellular cytotoxicity, but were resistant to infection by *Shigella sonnei*. When guinea-pigs were fasted before the opium tincture was administered, further decreases in both types of lymphocyte cytotoxicity were observed, and the lymphocytes

were susceptible to *Shigella sonnei* infection (Morgan et al., 1984).

4.2.4 Induces oxidative stress

See Table 4.5.

This section describes the effects of opium on oxidative stress and on antioxidants. Findings from four studies in exposed humans are described below. No data from studies in human cells in vitro or in other experimental systems were available to the Working Group.

There are several biomarkers that are used to assess oxidative stress in studies in humans. These biomarkers assess oxidative damage to DNA, protein oxidation, and lipid peroxidation in cellular systems. No studies on opium examining DNA damage by formation of 8-oxodeoxyguanosine were available to the Working Group. [The Working Group noted that formation of 8-oxodeoxyguanosine is the most studied and abundant oxidative DNA lesion (used as a specific biomarker of oxidative DNA damage), which is characterized by inducing G→T transversions, which are mutagenic.]

Ghazavi et al. (2013b) assessed redox status by measuring NO levels in serum samples from 44 male opium smokers and 44 healthy age-, sex-, socioeconomic status-, and tobacco-smoking status-matched controls with no lifetime history of drug abuse. NO production was estimated by the Griess reaction. Levels of NO in serum samples from opium smokers were higher than in those from the controls, but this increase was not statistically significant. Salarian et al. (2018) investigated plasma malondialdehyde levels, an index of lipid peroxidation, in 20 tobacco-smoking opium users attending community clinics and 40 (20 smoking and 20 non-smoking) healthy controls. Urine tests were conducted to confirm opium use, and users of other substances were excluded. Malondialdehyde levels (assayed via thiobarbituric acid-reacting substances) did not significantly differ between the opium-user and control groups. After an intervention (quitting opium and substituting with methadone), malondialdehyde levels significantly decreased by days 7 and 14 in the intervention group after methadone therapy, compared with before methadone therapy in tobacco-smoking opium users (Salarian et al., 2018).

The activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase were reported to be decreased in two studies (Safarinejad et al., 2013; Salarian et al., 2018). In the study described in the paragraph above, Salarian et al. (2018) reported that erythrocyte SOD activity (measured by the oxidation of NADP/NADPH) was lower in the 20 tobacco-smoking opium users than the 40 healthy controls (both the smoking and non-smoking groups), but the decrease was only significant when compared with the 20 non-smoking controls. Catalase activity (measured by decomposition of hydrogen peroxide) was significantly lower in patients with opioid use disorder and in the tobacco-smoking opium users and control groups than the non-smoking control group. After the 20 tobacco-smoking opium addicts received an intervention (quitting opium and substituting with methadone), levels of both SOD and catalase significantly increased by day 14 of the intervention (Salarian et al., 2018). Similarly low SOD and catalase levels in semen of opium users were reported by Safarinejad et al. (2013). This study compared 142 men who were opiate addicts with 146 men who were healthy controls. Significantly lower levels of SOD- and catalase-like activities were seen in addicts than in controls. [The Working Group noted that the latter study included 36 (25.3%) heroin users among the opiate addicts; however, Table 3 of the study reports similarly significant decreases in SOD- and catalase-like activities for users of crude and refined opium separately from the data for the heroin users.]

Total antioxidant capacity (TAC) was reported in two studies (Ghazavi et al., 2013b; Dwivedi

Table 4.5 Effects of opium use on oxidative stress markers in exposed humans

End-points	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
Redox status, TAC	Serum/ plasma	Arak, Iran (Islamic Republic of), exposed/ unexposed comparison	44 opium-addicted men from a detox centre (aged 20–40 yr; mean 31 yr) who smoked opium, > 2 g/day for ≥ 1 yr (range 2000–3000 mg); 44 age-, sex-, SES-, and tobacco-smoking status-matched controls	Serum levels of NO higher (92.90 ± 9.12 µM) in opium users compared with controls (83.92 ± 4.85 µM) but NS ($P = 0.344$) Higher FRAP values in opium users (972.75 ± 11.55 µM) compared with controls (761.95 ± 18.61 µM) ($P < 0.0001$)	NR	No details were given about how the opium history was obtained, probably by questionnaire. Well-defined exposure for consumption of opium (> 2 g/day for ≥ 1 yr), which was not further characterized; confirmed by urine tests; smoked as *teriak*; years of exposure not collected. Polydrug abusers and alcohol users excluded; tobacco smokers included.	Ghazavi et al. (2013b)
Lipid peroxidation (MDA) and antioxidant enzymes (SOD and CAT activity)	Plasma, erythrocytes	Mazandaran, Iran (Islamic Republic of), exposed/ unexposed comparison	20 tobacco-smoking opium addicts (> 1 g/day for ≥ 1 yr); 40 controls (20 smokers/20 non-smokers)	MDA level not significantly different Lower SOD activity in patients before methadone therapy (12.71 ± 10.005 U/mg haemoglobin) compared with the smoker (20.08 ± 10.34; NS) or non-smoker (25.18 ± 11.25) comparison group ($P < 0.05$) Lower CAT activity in the patients with opioid use disorder (224.56 ± 37.7 k/mL) and in tobacco-smoking group (216.82 ± 33.4 k/mL) compared with the non-smoker (274.22 ± 31.6 k/mL) group (both $P < 0.05$)	NR	Data collected at clinical interview; opium use validated by opioid in urine at baseline. Well-defined, validated exposure, unclear if collected intensity and duration except to confirm minimum exposure; type of opium and method of exposure not presented; comparison group clearly unexposed to opium. Other substance users excluded by urine tests.	Salarian et al. (2018)

Table 4.5 Effects of opium use on oxidative stress markers in exposed humans (continued)

End-points	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
Antioxidant enzymes (SOD and CAT)	Semen	Iran (Islamic Republic of); patients from several addiction treatment centres; exposed/ unexposed comparison	142 male opiate users (age, 20–50 yr) selected by DSM-IV-TR criteria for addiction, reporting use of 2.7 ± 1.2 nokhods[a]/ day; 146 healthy male controls	Lower SOD-like activity in opium users (38.4 ± 1.4 U/mL) compared with controls (49.3 ± 12.2 U/mL) ($P = 0.002$) Lower CAT-like activity in opium users (316 ± 17 U/mL) compared with controls (371 ± 42 U/mL) ($P = 0.003$)	Age, BMI, occupational status, educational level, smoking status, serum testosterone, luteinizing hormone, and prolactin	Could be questionnaire or interview; opium use validated by opioid in urine, but results not presented and no individual results for opium-only use presented. Well-defined, apparently validated exposure; collected intensity and duration, type of opium, and method of exposure; cannot separate opium and heroin users. Polydrug consumers excluded by urine analysis. 36 of the opiate users were heroin users; similar results excluding the heroin users. Tobacco smokers included.	Safarinejad et al. (2013)

Table 4.5 Effects of opium use on oxidative stress markers in exposed humans (continued)

End-points	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
TAC	Serum	Rajasthan, India; addiction clinic of a tertiary care centre; exposed/unexposed comparison	90 male opiate users (≥ 100 mg/day for ≥ 1 yr) and 30 healthy controls	TAC higher in opiate-only users while opiate users who smoked tobacco had significantly lower TAC	Age, dependence years, and basic biochemical profile	"Chronic opiate abusers" diagnosed with ICD-10, recruited from addiction clinic; controls were their attendees; screened by urine opiate test; no information about type of opium or tobacco exposure was collected; severity of opiate dependence evaluated by SODQ Opium use ≥ 100 mg/day for ≥ 1 yr duration described as "chronic"; types of opium exposure combined (pure opium, opium husk, and includes heroin); included opium-only users group and opium + tobacco-smoking and chewing tobacco groups; multiple-substance abusers excluded. Well-defined exposure to opiates (note this included opium or heroin), which was not further characterized; opiate use ≥ 100 mg/day for ≥ 1 yr, duration described as "chronic"; types of opium exposure combined (pure opium, opium husk, and includes heroin); smoking and chewing tobacco, but no other exposures considered.	Dwivedi et al. (2019) See also Purohit et al. (2017)

Table 4.5 Effects of opium use on oxidative stress markers in exposed humans (continued)

End-points	Sampling matrix	Location, setting, study design	Exposure level and number of exposed and controls	Response (significance)	Covariates controlled	Comments	Reference
Lipid peroxidation (MDA), protein oxidation (protein carbonyl), antioxidant enzymes (SOD activity), and TAC	Serum	Afzalipour and Shafa, and Payambar-e-Azam hospitals, Kerman, Iran (Islamic Republic of); admissions for lead poisoning; exposed/unexposed comparison	192 opium addicts (median use 2 g/day) with symptoms of lead poisoning and 104 healthy controls with no occupational contact with lead	MDA level significantly higher in opium addicts (0.45 ± 0.3 µM) compared with controls (0.17 ± 0.16 µM) ($P < 0.001$) Protein carbonyl significantly higher in opium addicts (0.31 ± 0.09 mM) compared with controls (0.19 ± 0.09 mM) ($P < 0.001$) Significantly lower SOD activity in opium addicts (7.6 ± 1.4 U) compared with controls (28.7 ± 5.1 U) ($P < 0.001$) TAC significantly lower (0.24 ± 0.22 mM) in opium addicts compared with controls (1.04 ± 0.15 mM) ($P < 0.001$)	None	Data collection (a structured interview with questionnaire). Opium exposure defined as "addicts"; duration of exposure undefined. Route of opium use 16% inhalation, 62% oral, and 22% both. Lead co-exposure (lead-adulterated opium), and evidence of lead poisoning in the opium users. 48% of opium users smoked tobacco; smoking in unexposed group unknown.	Shojaeepour et al. (2018)
Interventions (7–14 days)							
Lipid peroxidation (MDA) and antioxidant enzymes (SOD and CAT)	Plasma Erythrocytes		20 opium addicts who quit opium use and substituted with methadone	MDA was significantly decreased from baseline on days 7 and 14 ($P < 0.05$) CAT levels significantly improved by day 7 and SOD levels by day 14 of the intervention ($P < 0.05$)		See Salarian et al. (2018) above. Quitting opium use had an immediate significant effect on oxidative stress when tested after 7 or 14 days.	Salarian et al. (2018)

BMI, body mass index; CAT, catalase; DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision; FRAP, ferric reducing/antioxidant power; ICD-10, International Classification of Diseases 10th Revision; MDA, malondialdehyde; NO, nitric oxide; NR, not reported; NS, not significant; SES, socioeconomic status; SOD, superoxide dismutase; SODQ, severity of opiate dependence questionnaire; TAC, total antioxidant capacity; yr, year.

[a] *Nokhod*, the local unit for opium use, approximately 0.2 g.

et al., 2019). In the study described above, Ghazavi et al. (2013b) examined TAC using the ferric reducing/antioxidant power (FRAP) test. FRAP levels were significantly higher in the 44 opium smokers than in the 44 controls, suggesting that opium smoking increased the antioxidant capacity. Dwivedi et al. (2019) conducted a study of 90 chronic opiate users who were men attending an addiction centre and 30 healthy controls in Rajasthan, India, which was validated by urine tests, and measured TAC using the 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate) method. [The Working Group noted that the test method was stated in a publication by the same group (Purohit et al. (2017).] The opiate users were subdivided into three groups: opium-only users, opium users who chewed tobacco, and opium users who smoked tobacco. They reported that opium-only users had higher TAC levels than the controls.

Shojaeepour et al. (2018) reported significantly higher malondialdehyde and protein carbonyl (a marker of protein oxidation) levels, lower SOD activity, and lower TAC in 192 opium addicts with clinical signs of lead poisoning from lead-adulterated opium compared with 104 controls with no occupational exposure to lead.

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