Chemosphere 214 (2019) 534–542

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

A mixture of Persistent Organic Pollutants (POPs) and Azoxymethane (AOM) show potential synergistic effects on intestinal tumorigenesis in the A/J Min/+ mouse model

K.E.Aa Hansen [a, *], S.M. Johanson [a], C. Steppeler [b], M. Sødring [b, g], G.C. Østby [c], H.F. Berntsen [c, d], K.E. Zimmer [e], M. Aleksandersen [f], J.E. Paulsen [b], E. Ropstad [a]

[a] Section for Experimental Biomedicine, Department of Production Animal Clinical Sciences, Norwegian University of Life Sciences, Norway
[b] Section for Food Safety, Department of Food Safety and Infection Biology, Norwegian University of Life Sciences, Norway
[c] Section for Stationary Clinics, Department of Production Animal Clinical Sciences, Norwegian University of Life Sciences, Norway
[d] Department of Administration, Laboratory Animal Unit, National Institute of Occupational Health, Norway
[e] Section for Biochemistry and Physiology, Department of Basic Sciences and Aquatic Medicine, Norwegian University of Life Sciences, Norway
[f] Section for Anatomy and Pathology, Department of Basic Sciences and Aquatic Medicine, Norwegian University of Life Sciences, Norway
[g] Animalia, Norwegian Meat and Poultry Research Centre, Norway

HIGHLIGHTS

- Can a mixture of POPs affect intestinal tumorigenesis in the A/J Min/+ mouse?
- Mice were exposed to POPs through the diet and received an injection of Azoxymethane.
- Results show an increased intestinal tumorigenesis in the A/J Min/+ mouse model.

ARTICLE INFO

Article history:
Received 5 July 2018
Received in revised form
19 September 2018
Accepted 20 September 2018
Available online 24 September 2018

Handling Editor: A. Gies

Keywords:
Persistent Organic Pollutants
Colorectal cancer
A/J Min/+ mouse
Azoxymethane
Intestinal tumorigenesis
Synergistic effect

ABSTRACT

A multitude of cancer types, including breast, testicular, liver and colorectal cancer, have associations with exposure to Persistent Organic Pollutants (POPs). The present study aimed to investigate whether a mixture of POPs could affect intestinal tumorigenesis in the A/J Min/+ mouse, a model for human colorectal cancer (CRC). Pollutants were selected for their presence in Scandinavian food products and the mixture was designed based on defined human estimated daily intake levels. Mice were exposed through the diet, at control, low and high mixture concentrations, for 10 weeks. In a separate experiment, mice also received one subcutaneous injection of Azoxymethane (AOM) to explore whether this carcinogenic compound influenced the effect of the POPs. Intestinal tumorigenesis was examined by surface microscopy and histopathology. Moderate and dose-dependent increases in tumorigenesis were observed after dietary POP exposure. The AOM treatment alone stimulated the growth of colonic lesions, but did not increase the formation of new lesions. Combined AOM treatment and POP exposure demonstrated a synergistic effect on lesion formation in the colon, and to a lesser extent in the small intestine. This synergy was also evident by an increased number of malignant colonic tumors (carcinomas). In conclusion, the study shows that a mixture of POPs interacted synergistically with a known carcinogen (AOM), causing increased intestinal tumorigenesis in the A/J Min/+ mouse model.

1. Introduction

Persistent Organic Pollutants (POPs) are man-made chemicals that are toxic to humans and wildlife, resistant to degradation and have the potential to bioaccumulate and biomagnify in living organisms (UNEP, 2015). The compounds have adverse health effects and have been associated with an increased risk of breast cancer (Hoyer et al., 2000; Cameron and Foster, 2009), testicular cancer (McGlynn et al., 2008; Giannandrea et al., 2011), liver cancer (Filgo et al., 2015), and colorectal cancer (Howsam et al., 2004; Song et al., 2014). The main route of non-occupational exposure to POPs in

* Corresponding author.
E-mail address: kristine.hansen@nmbu.no (K.E.Aa Hansen).

https://doi.org/10.1016/j.chemosphere.2018.09.126

humans is through ingestion (Darnerud et al., 2006; Vestergren et al., 2012), which makes the GI tract the first organ of exposure. Traditional animal experiments only assess the impact of POPs using single compounds (Sethi et al., 2017) or compounds belonging to the same chemical group (Colter et al., 2018). However, carcinogenesis is a multistep process, so focus on individual compounds may prevent the discovery of potential synergism between multiple chemicals.

Colorectal cancer (CRC) is the third most common cancer in humans worldwide and exposure to carcinogens through the diet is an essential risk factor (IARC, 2016). CRC develops as a result of several genetic and epigenetic changes that cause a transformation of intestinal epithelium from normal tissue, via benign neoplasms, into carcinomas (Kinzler and Vogelstein, 1996; Sancho et al., 2004). Up to 85% of CRC cases are considered sporadic and 1% are attributed to the hereditary CRC syndrome known as familial adenomatous polyposis (FAP) (Burt, 2000). Mutations in the tumor-suppressor gene adenomatous polyposis coli (*APC*) are responsible for FAP, and patients develop a vast number of adenomatous polyps in the intestine, which are likely to progress into malignant tumors (Kinzler and Vogelstein, 1996). In addition, dysfunctional *APC* alleles have been found in the majority of sporadic colorectal lesions (Fodde, 2002). Research on CRC caused by *APC* mutations is therefore highly relevant to human health.

The most widely used animal model for human CRC is the multiple intestinal neoplasia (Min/+) mouse. This mouse has a heterozygous mutation in the *Apc* gene, resulting in a truncated gene product at amino acid 850 (Su et al., 1992). Inactivation of the remaining functional allele in the intestinal epithelium appears to be the rate-limiting step in tumorigenesis (Luongo et al., 1994). Loss of *Apc* inhibits the formation of the β-catenin destruction complex, leading to accumulation of β-catenin in the cytoplasm and subsequent translocation to the nucleus. Here, it interacts with the transcription factor Tcf-4, creating an active complex that transcribes specific target genes (Fodde, 2002; Kretzschmar and Clevers, 2017). The conventional Min/+ mouse model, bred on a C57BL/6 genetic background (Moser et al., 1990), develops lesions primarily in the small intestine (Mollersen et al., 2004). The A/J Min/+ mouse, on the other hand, also develops a large number of lesions in the colon, many of which progress to carcinomas over time (Sødring et al., 2016b). Therefore, the A/J Min/+ mouse model more closely resembles CRC development in humans and was therefore chosen for the present study.

The A/J strain has been shown to be more susceptible to the induction of colorectal cancer by Azoxymethane (AOM) than its C57BL/6 counterpart (Nambiar et al., 2003; Meunier et al., 2011). AOM is a genotoxic chemical used to mimic sporadic CRC and to study the underlying mechanisms of sporadic colorectal carcinogenesis (Venning FA, 2013). Following metabolic activation by cytochrome P450 enzymes (mostly CYP2E1), AOM reacts with DNA and causes adduct formation, leading to DNA mutations initiating colorectal carcinogenesis (Takahashi and Wakabayashi, 2004).

The aim of this study was to investigate whether dietary POP exposure, alone or following AOM treatment, could affect intestinal tumorigenesis in the A/J Min/+ mouse model. The mixture was designed to simulate a real-life exposure scenario relevant to humans (Berntsen et al., 2017).

2. Animals, materials and methods

2.1. Ethics statement

The study was performed at the Section for Experimental Biomedicine at The Norwegian University of Life Sciences in Oslo, Norway. The animal facility is licensed by the Norwegian Food Safety Authority (https://www.mattilsynet.no/language/english/) and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (https://www.aaalac.org/). The animal experiment was approved by the unit's animal ethics committee (Institutional Animal Care and Use Committee/IACUC) and the Food Safety Authority (application ID: FOTS 8127) and executed in compliance with the local and national regulations associated with laboratory animal experiments. The rodent and rabbit section of the facility is a Specific Pathogen Free (SPF) unit and follows a health monitoring program recommended by Federation of European Laboratory Animal Science Associations/FELASA (http://www.felasa.eu/). The care of the animals was carried out by two veterinary nurses with FELASA B certification and the study was performed by a veterinarian with FELASA C certification.

2.2. Chemicals and experimental diet

A thorough description of the design and preparation of the POP mixture can be found in Berntsen et al. (2017). A list of the individual compounds can be found in Table 1. In brief, compounds occurring in Scandinavian food products reported in studies prior to 2012 were selected for the POP mixture. Human estimated daily intake (hEDI) levels were defined and adjusted to a 25 g mouse consuming 3 g feed/day. However, due to the possibility of background exposure and interspecies differences in compound metabolism, concentrations were adjusted up to 5000× (low dose) and 100 000× (high dose) hEDI. All polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and other organochlorines were purchased from Chiron AS (Trondheim, Norway). All perfluorinated compounds (PFCs) and hexabromocyclododecane (HBCD) were obtained from Sigma-Aldrich (St. Louis, MO, USA), with the exception of perfluorohexane sulfonic acid (PFHxS) potassium salt which was purchased from Santa Cruz (Dallas, US). All chemicals were dissolved in an appropriate solvent and added to corn oil (Jasmin, fully refined, Yonca Gida San A.S., Manisa, Turkey) intended for human consumption. Solvents were thoroughly evaporated under N_2-flow and the remaining oil was incorporated in AIN-93G mouse feed (TestDiets, St.Louis, MO) at the low and high mixture concentrations. The control diet contained only corn oil from which the solvent had been evaporated.

2.3. Study design

In Experiment 1, 66 mice were used and each litter was randomly divided into 3 exposure groups (control, low and high POP diet) at weaning and exposed for 10 weeks (Fig. 1). In Experiment 2, 21 mice were exposed to the mixture of POPs in the same way, but in addition, these mice were also given one subcutaneous injection of 8.5 mg/kg AOM (Sigma-Aldrich, St. Louis, MO, USA) during their second week after birth. After 10 weeks of POP exposure, all mice were sacrificed and sampled. Because of high offspring mortality after the AOM injection, the breeding of mice for Experiment 2 was terminated for animal welfare reasons prior to completion of breeding the individuals for the study. This resulted in a lower number of animals compared to Experiment 1.

2.4. Animal model

The A/J Min/+ mouse model was established by backcrossing the Min/+ trait onto the genetic background of the A/J strain for >12 generations (Sødring et al., 2016b). In the present study, a total of 87 A/J Min/+ mice were used. The animals were bred in-house. Female A/J +/+ mice were mated with male A/J Min/+ mice and their A/J Min/+ offspring were used in the present study. The pups were marked with ear punches and genotyped at weaning, as

Table 1
A mixture of persistent organic pollutants (POPs) based on a literature review on estimated daily intake (EDI) values in the Scandinavian population (Berntsen et al., 2017). Average EDI values for a 70 kg human and corresponding values for a 25 g mouse are shown. EDI values for a 25 g mouse consuming 3 g of feed designed to provide daily doses of POPs corresponding to the low (5000× human EDI) and high (100,000× human EDI) doses are shown in grey, and are based on measured feed concentrations. The table is adapted from Berntsen et al. (2017).

Compound	Average EDI [a]	Daily intake human	EDI [b] 25 g	EDI [c] 25 g mouse	EDI [d] 25 g mouse	Feed measured [e]	Feed measured [f]	EDI [g] 25 g mouse	EDI [h] 25 g mouse
	70 kg person ng/day	ng/kg/day	mouse pg/day	5000× ng/day	100,000× ng/day	5000× ng/g feed	100,000× ng/g feed	5000× ng/day	100,000× ng/day
Chlorinated									
PCB 28	10	0.14	3.5	18	350	3.1	46	9	138
PCB 52	23	0.33	8.3	41	825	15.0	182	45	546
PCB 101	39	0.56	14.0	70	1400	25.4	377	76	1131
PCB 118	68	0.97	24.3	121	2425	37.2	612	112	1836
PCB 138	97	1.38	34.5	173	3450	53.8	957	161	2871
PCB 153	97	1.38	34.5	173	3450	61.4	981	184	2943
PCB 180	26	0.37	9.3	46	925	17.4	263	52	789
∑PCBs	360	5.13	128.4	642	12,825	213.3	3418	640	10,254
p,p'-DDE	201	2.87	71.8	359	7175	136.0	2390	408	7170
HCB	84	1.20	30.0	150	3000	37.4	588	112	1764
a-Chlordane	63	0.90	22.5	113	2250	45.0	723	135	2169
Oxychlordane	21	0.30	7.5	38	750	9.8	297	29	891
trans-Nonachlor	21	0.30	7.5	38	750	14.9	264	45	792
a-HCH	36	0.52	13.0	65	1300	21.2	421	64	1263
b-HCH	29	0.42	10.5	53	1050	22.3	398	67	1194
g-HCH (Lindane)	40	0.57	14.3	71	1425	31.4	435	94	1305
Dieldrin	126	1.80	45.0	225	4500	70.4	1470	211	4410
∑OCPs	621	8.88	222.1	1112	22,200	388.4	6986	1165	20,958
∑PCBs + OCPs	981	14.01	350.5	1754	35,025	601.7	10,404	1805	31,212
Brominated									
PBDE 47	68	0.97	24.3	121	2425	39.7	642	119	1926
PBDE 99	13	0.19	4.8	24	475	8.6	126	26	378
PBDE 100	11	0.15	3.8	19	375	5.6	91	17	272
PBDE 153	2	0.03	0.8	4	75	1.5	22	5	67
PBDE 154	4	0.06	1.5	8	150	2.8	38	8	114
PBDE 209	105	1.50	37.5	188	3750	64.8	1141	194	3423
HBCD	21	0.30	7.5	38	750	9.9	203	30	609
∑BFRs	224	3.2	80.2	402	8000	132.9	2263	399	6789
Perfluorinated									
PFHxS	1.2	0.017	0.4	2	43	1.7	42	5	125
PFOS	18	0.26	6.5	33	650	3.2	74	10	222
PFOA	31	0.44	11.0	55	1100	6.0	121	18	363
PFNA	9.5	0.14	3.5	18	350	2.1	42	6	127
PFDA	13	0.19	4.8	24	475	3.1	57	9	172
PFUnDA	6.7	0.096	2.4	12	240	1.6	28	5	84
∑PFAAs	79.4	1.14	28.6	144	2858	17.7	364	53	1094

Abbreviations: PCBs (polychlorinated biphenyls); OCPs (organochlorine pesticides); BFRs (brominated flame retardants); PFAAs (perfluoroalkyl acids).
[a] Average EDI (Estimated daily intake) values of POPs for a 70 kg human e based on a literature review of Scandinavian EDI values (Berntsen et al., 2017).
[b] EDI values for a 25 g mouse corresponding to human EDI values.
[c] EDI values for a 25 g mouse corresponding to human EDI values * 5000
[d] EDI values for a 25 g mouse corresponding to human EDI values * 100,000.
[e] Measured concentrations of the various compounds in the 5000× feed.
[f] Measured concentrations of the various compounds in the 100,000× feed.
[g] EDI values for a 25 g mouse consuming 3 g of the 5000× feed/day e based on concentrations measured in the feed of the current project.
[h] EDI values for a 25 g mouse consuming 3 g of the 100,000× feed/day e based on concentrations measured in the feed of the current project.

previously described in Sødring et al. (2015).

2.5. Housing and husbandry

During mating, animals were housed in groups in open type III cages (Tecniplast, Buguggiate, Italy). During exposure and AOM injection animals were housed in closed type III IVC-cages (Allentown Inc, USA) for health and safety reasons. All cages contained standard aspen bedding, cellulose nesting material and red polycarbonate houses (Tecniplast, Buguggiate, Italy). The animals were given their assigned feed, and tap water in standard drinking bottles (Tecniplast, Buguggiate, Italy), *ad libitum*. The animal room was on a 12:12 light–dark cycle, with a room temperature of 21 ± 2 °C with 20 air changes per hour and 45 ± 5% relative humidity. The cages, bedding, nesting material and water bottles were changed once a week.

2.6. Sample collection and identification of intestinal lesions

The A/J Min/+ offspring were sacrificed at 13 weeks of age. They were anesthetized with isoflurane gas (Isoflurane Baxter, San Juan, Puerto Rico), bled by cardiac puncture and euthanized by cervical dislocation. The small intestine and colon were collected, fixed and dyed as previously described in Sødring et al. (2016a). Briefly, the intestines were rinsed with PBS, fixed flat, and stored in 10% neutral buffered formalin for at least 24 h, before being stained with 0.2% methylene blue dissolved in formalin. The liver was collected and weighed. All tumors that were found (one in the liver, one from the

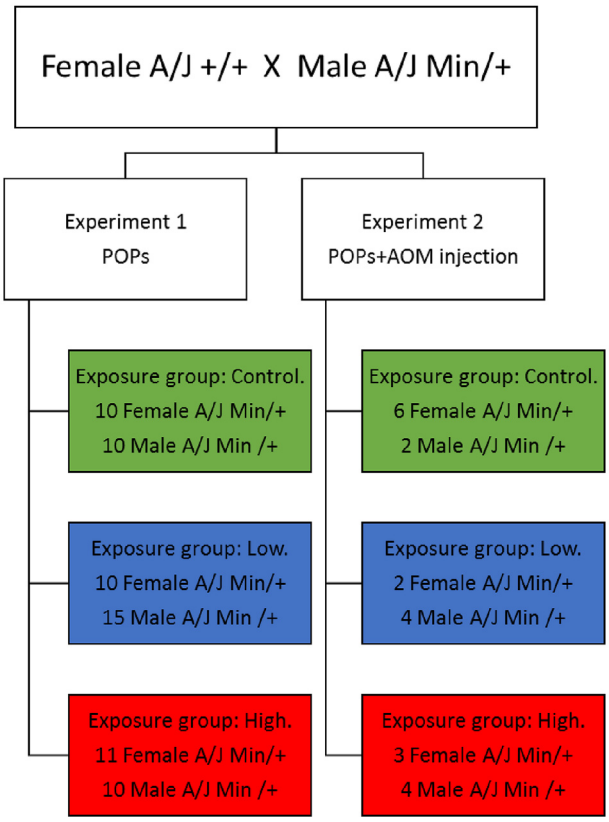

Fig. 1. Study design of the two experiments, including exposure groups (control, low and high), breeding of A/J Min/+ mice and the number of animals (females and males) in each group. In both experiments, A/J Min/+ mice were exposed to a mixture of POPs through feed for 10 weeks. In addition, mice in Experiment 2 received one subcutaneous injection of AOM (8.5 mg/kg) during the second week after birth.

forelimb, one sub-mandibular and one from the abdomen) were also collected and fixed in 10% neutral buffered formalin. The blood, cecum, spleen and retroperitoneal adipose tissue were collected and stored for analysis in another project. For surface microscopy and transillumination of the intestines, an inverted light microscope (CKX41, Olympus Inc., Hamburg, Germany) with a digital color camera (DP25, Olympus) was used. In the colon, lesions were identified as either flat aberrant crypt foci (flat ACF; <30 crypts) or tumors (>30 crypts covering more than approximately 0.4 mm^2) as explained by Sødring et al. (2015).

2.7. Histology

After scoring, the intestines were prepared using the Swiss roll technique as described earlier by Sødring et al. (2016b). The Swiss rolls were embedded in paraffin and 3 μm thick histological sections were cut and stained with haematoxylin eosin (HE) and periodic acid Schiff (PAS). All Swiss rolls were sectioned at three different random levels in the paraffin block. Examination was conducted in a microscope and lesions were identified, counted and classified as preneoplastic lesions (hyperplastic and dysplastic cells), adenomas or carcinomas. Tumors with distinct infiltrative growth through the muscularis mucosa and into the submucosa were classified as carcinomas, whereas tumors confined to the mucosa without infiltrative growth were classified as adenomas. Tumors that were found outside the intestine were also embedded in paraffin, sectioned and stained with HE and PAS, and examined in the microscope.

2.8. Statistical analyses

Statistical analyses were performed using JMP Pro 13® (SAS, Cary, NC, USA). Least squares analyses were used to analyze data on body measures. Experiment 1 and 2 were analyzed separately by the following model:

$$Y_{ijpmn} = \mu + G_i + E_j + e_{ij}$$

where:

Y_{ij} = observation of either body weight, relative liver weight, relative colon length or relative small intestine length.
μ = overall mean of body weight, relative liver weight, relative colon length and relative small intestine length.
G_j = effect of sex, $i = 1$ (Male) or 2 (Female).
E_j = effect of exposure group, $j = 1$ (control), $j = 2$ (low), $j = 3$ (high).
e_{ij} = error term.

Measures of histological changes and visually scored lesions did not meet the assumption of normality. Log transformation provided an improved, but not satisfactory, fit to the normal distribution. Initially least squares analyses were performed on log-transformed data with sex and exposure group as explanatory variables. Some sex differences were noticed, but few interactions were found between the exposure group and the sex of the animal. Thus, exposure effects were not dependent on the sex. In the final analyses, univariate non-parametric tests were used. Differences between exposure groups and the control were assessed using Steel's test, which controls for the overall experiment wise error rate (Type I). Differences between sexes were investigated using the Wilcoxon two-sample test. The level of significance was set to 5%. Size and location distribution figures were produced using Excel 2013®.

3. Results

3.1. Effects on body weight, liver weight and intestinal length

The high mixture concentration of POPs significantly decreased the terminal body weight of both the mice who only were exposed to POPs (Experiment 1) and also the mice that were injected with AOM (Experiment 2), compared to the control group (Table 2). In addition, there was a significant increase in liver weight, relative to body weight, in the high group of both experiments. Colon length, relative to body weight, was not affected by AOM or POPs. However, the length of the small intestine was significantly increased by the high concentration of POPs after the AOM injection (Experiment 2). Notably, AOM alone did not change any of the parameters measured.

3.2. Scoring of intestinal lesions

The effects of dietary exposure to the mixture of POPs on intestinal tumorigenesis was examined in mice by scoring of intestinal lesions (Table 3). High levels of POPs (Experiment 1) significantly increased the number of flat ACF in colon, when compared to the control group. Although not significant, a trend was observed towards increased flat ACF load in the high and the low groups ($p = 0.051$ and $p = 0.058$, respectively). The low mixture concentration increased the number of colonic tumors, compared to the control group. However, this was not evident after exposure to the high mixture concentration ($p = 0.096$). No other parameters measured in the small intestine and colon were affected by dietary POPs alone.

Table 2
Least square mean (±SE) of body weight (BW), relative liver weight (LW), relative colon length and relative small intestine (SI) length at necropsy in Experiment 1 (POP exposure) and Experiment 2 (POP exposure + AOM injection). The table included effects of exposure group control, low and high in both experiments. Bold letters indicate significant difference from the control group (Dunnet's test; $p \leq 0.05$).

Exposure		BW at necropsy (g)	Relative LW	Relative colon length (cm/g)	Relative SI length (cm/g)
Experiment 1	Control	23.54 ± 0.71	$0.05 \pm 0.08 \times 10^{-2}$	0.31 ± 0.01	1.41 ± 0.03
POPs	Low	23.26 ± 0.79	$0.05 \pm 0.07 \times 10^{-2}$	0.31 ± 0.01	1.46 ± 0.03
	High	$\mathbf{21.47 \pm 0.49}$	$\mathbf{0.07 \pm 0.08 \times 10^{-2}}$	0.33 ± 0.01	1.49 ± 0.03
Experiment 2	Control	24.68 ± 1.09	$0.05 \pm 0.12 \times 10^{-2}$	0.31 ± 0.01	1.36 ± 0.05
POPs + AOM	Low	25.43 ± 1.44	$0.05 \pm 0.14 \times 10^{-2}$	0.31 ± 0.03	1.49 ± 0.05
	High	$\mathbf{20.99 \pm 1.47}$	$\mathbf{0.07 \pm 0.13 \times 10^{-2}}$	0.33 ± 0.02	$\mathbf{1.62 \pm 0.05}$

Table 3
Summary of results from scoring of lesions in colon and small intestine (SI) of A/J Min/+ mice from Experiment 1 (POP exposure) and Experiment 2 (POP exposure + AOM injection). Colonic lesions are categorized as either flat ACF (<30 aberrant crypts) or tumors (>30 aberrant crypts). Load equal the total area of intestine covered by lesions. Results are presented as means (±SE). Differences between exposed groups (low and high) and control were assessed with Steel's test and indicated in bold when significant ($p \leq 0.05$). Trends with $p \leq 0.07$ are denoted *.

Exposure		Colon						SI		
		Number of flat ACF	Average size of flat ACF (mm^2)	Load of flat ACF (mm^2)	Number of tumors	Average size of tumors (mm^2)	Tumor load (mm^2)	Number of tumors	Average size of tumors (mm^2)	Tumor load (mm^2)
Experiment 1 POPs	Control	4.75 ± 0.98	$0.01 \pm 0.03 \times 10^{-1}$	0.06 ± 0.02	0.70 ± 0.24	0.75 ± 0.23	1.07 ± 0.37	27.95 ± 5.12	0.62 ± 0.04	20.39 ± 4.77
	Low	8.60 ± 1.97	$0.02 \pm 0.04 \times 10^{-1}$	$0.18^{*} \pm 0.04$	$\mathbf{2.04 \pm 0.47}$	0.65 ± 0.15	2.52 ± 0.93	25.56 ± 4.50	0.58 ± 0.04	17.92 ± 4.76
	High	$\mathbf{8.38 \pm 1.42}$	$0.02 \pm 0.02 \times 10^{-1}$	$0.13^{*} \pm 0.03$	1.24 ± 0.23	0.47 ± 0.11	0.81 ± 0.23	26.57 ± 3.76	0.55 ± 0.03	15.85 ± 3.51
Experiment 2 POPs + AOM	Control	2.25 ± 0.84	0.05 ± 0.02	0.15 ± 0.06	3.63 ± 0.78	0.77 ± 0.20	3.47 ± 1.37	18.75 ± 5.44	0.70 ± 0.03	13.70 ± 4.39
	Low	6.17 ± 1.30	0.06 ± 0.01	0.35 ± 0.09	12.33 ± 3.33	1.72 ± 0.50	25.80 ± 9.67	18.33 ± 2.33	0.63 ± 0.05	11.52 ± 1.57
	High	$\mathbf{11.57 \pm 2.03}$	0.06 ± 0.01	$\mathbf{0.69 \pm 0.15}$	$\mathbf{29.86 \pm 4.83}$	$\mathbf{1.99 \pm 0.17}$	$\mathbf{56.62 \pm 6.70}$	$\mathbf{48.00 \pm 9.85}$	0.74 ± 0.10	40.85 ± 13.44

In combination with the AOM injection (Experiment 2), POPs significantly increased the number of flat ACF, flat ACF load, number of tumors, average tumor size and tumor load in the colon of mice in the high group (Table 3). In addition, the number of tumors in the small intestine was significantly higher in the high group compared to the controls. No significant changes were observed after exposure to the low mixture concentration of POPs in Experiment 2. However, there were clear trends towards increases in several parameters, including a 7-fold increase in the colonic tumor load.

3.3. Size distribution and location of intestinal lesions

To demonstrate the distribution of size, lesions were divided into five different size categories. Fig. 2a presents the number of lesions per size category for each exposure group in both experiments and clearly illustrates the shift towards larger lesions observed in AOM treated animals. The AOM treatment alone did not appear to give any new lesions, but instead stimulated the growth of the colonic lesions. Notably, the increase in the number of lesions provoked by POPs seemed to be more pronounced in

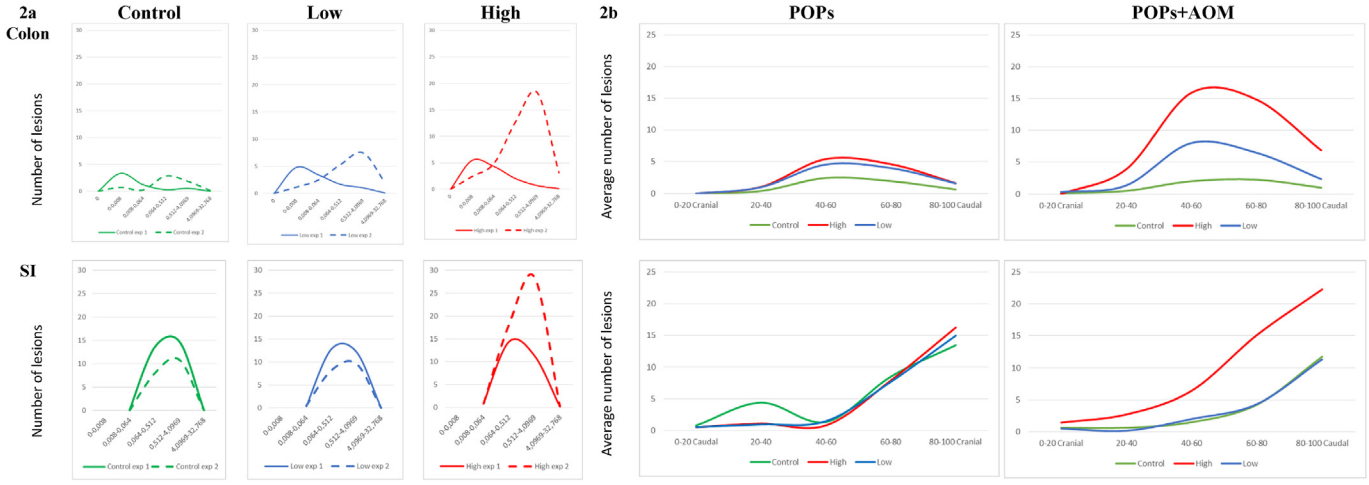

Fig. 2. a). Size distribution of lesions in colon and small intestine (SI) of A/J Min/+ mice exposed to POPs in control, low and high mixture concentrations, without AOM (Exp 1) and with AOM injection (Exp 2). Size categories (mm^2) are described by Sødring et al 2015 and represented on the X axis. The Y axis shows the number of lesions. b). Location of lesions in colon and small intestine (SI) of A/J Min/+ mice exposed to POPs in control, low and high mixture concentrations, without AOM (Exp 1) and with AOM injection (Exp 2). Location categories (20% sections) are represented on the X axis. The Y axis shows the average number of lesions.

AOM treated animals than in untreated animals, particularly in the colon. This implies a synergistic effect of AOM and POPs. In the small intestine, AOM alone did not induce any apparent changes, but a moderate synergistic effect on tumor formation seemed to occur between AOM and the high level of POPs.

Location of lesions along the intestine (Fig. 2b) shows an increased number of lesions in the middle and caudal parts of the colon and caudally in the small intestine, in both experiments. In addition, the figure illustrates how dietary exposure to POPs enhances the number of lesions in both experiments, represented by more lesions in the high and low groups compared to the control group.

3.4. Histopathology

Histology from tumors collected from non-intestinal tissue showed no metastases originating from the intestinal lesions. Instead, they were either hyperplastic lesions or metastases from the local tissue.

In Experiment 1, lesions were found in the intestines of animals from all exposed groups. The total number of lesions in the small intestine was higher than that of the colon (Table 4). No significant differences were observed between the control group and the exposed groups. Preneoplastic changes and adenomas were the most frequent lesions, and only a few animals had carcinomas. Fig. 3 illustrates the types of lesions in the colon in Experiments 1 and 2.

In Experiment 2, the mice fed the high concentration of POPs had significantly more colonic lesions of all types compared to the control group (Table 4). A trend was also evident towards increases in the number of small intestinal preneoplastic lesions ($p = 0.067$). Interestingly, this increase of lesions appeared to be due to the synergistic effect between AOM and the high level of POPs, as suggested above.

4. Discussion

In the present study, we investigated whether a mixture of POPs could affect intestinal tumorigenesis in the A/J Min/+ mouse model. In a separate experiment, we also investigated whether a sub-carcinogenic exposure of AOM could influence the effect of POPs. We found that POPs alone increased the intestinal tumorigenesis moderately and in a dose-dependent manner. Comparing the two experiments, AOM alone did not seem to increase the formation of new lesions, or have a deleterious effect on the mice. However, the growth of colonic lesions was stimulated by AOM treatment. A strong synergistic effect was apparent between POPs and AOM on the formation of colonic lesions, and to a lesser extent

Table 4
Histopathological examination of lesions (preneoplastic, adenoma or carcinoma) in colon and small intestine (SI) of A/J Min/+ mice from Experiment 1 (POP exposure) and Experiment 2 (POP exposure + AOM injection). Results are presented as mean (±SE). Differences between exposed groups (low and high) and control were assessed with Steel's test and indicated in bold when significant ($p \leq 0.05$). Trends with $p \leq 0.07$ are denoted *.

Exposure		Colon			SI		
		Preneoplastic	Adenoma	Carcinoma	Preneoplastic	Adenoma	Carcinoma
Experiment 1	Control	0.70 ± 0.25	0.35 ± 0.25	0.05 ± 0.05	9.05 ± 1.32	6.80 ± 1.33	0.90 ± 0.42
POPs	Low	0.84 ± 0.29	0.32 ± 0.19	0.12 ± 0.09	7.00 ± 1.21	3.96 ± 1.04	0.44 ± 0.22
	High	1.05 ± 0.30	0.05 ± 0.05	0.00 ± 0.00	6.86 ± 1.58	3.86 ± 1.09	0.29 ± 0.16
Experiment 2	Control	1.38 ± 0.42	0.75 ± 0.31	0.00 ± 0.00	4.50 ± 1.59	5.13 ± 1.75	0.50 ± 0.27
POPs + AOM	Low	2.00 ± 0.58	2.17 ± 0.70	0.67 ± 0.49	6.00 ± 1.21	2.67 ± 0.67	0.00 ± 0.00
	High	**5.57 ± 1.11**	**6.57 ± 1.51**	**2.57 ± 0.92**	10.29 * ± 2.35	9.43 ± 2.06	0.71 ± 0.36

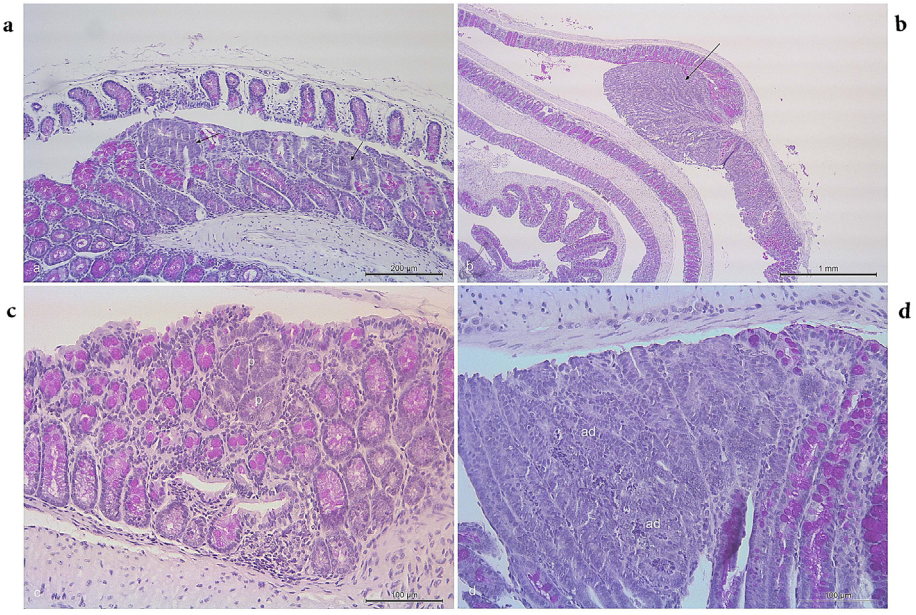

Fig. 3. Histological lesions observed in colon. a. Preneoplastic lesions (dysplasia and hyperplasia) are present in the luminal part of crypts (arrows) of a mouse of the low exposure group of Experiment 1. **b**. A carcinoma (arrow) in the mucosa infiltrates Muscularis Mucosae and Submucosa. Mouse of the low exposure group of Experiment 1. **c**. A small focus with preneoplastic crypt lesions in a mouse of the low exposure group of Experiment 2. **d**. Mucosal adenoma (ad) in a mouse from the high exposure group of Experiment 2.

on lesions in the small intestine. Interestingly, this synergy was also associated with a significant increase of malignant tumors (carcinomas) in the colon.

4.1. Effects of POPs on body weight and liver weight

The concentration of each compound in the high dose was generally below the No Observed Adverse Effect Level (NOAEL), where such a level was available (Berntsen et al., 2017). Although we did not observe any clinical signs in the animals during the present study, we did observe apparent adverse effects at the end of the study, indicated by reduced body weight and increased relative liver weight in both experiments. These effects were seemingly unrelated to AOM treatment and may have been caused by additive or synergistic effects between individual POPs in the mixture. In another experiment using the same mixture but a different mouse strain (129:C57BL/6F0), there was no significant effect of the high POPs feed on body weight (Hudecova et al., 2018). This suggests there are mouse strain differences in sensitivity to POPs.

Aberrant *Apc* expression as a consequence of the germline mutation in *Apc* has been shown to affect the ability of the liver to metabolize xenobiotics (Benhamouche et al., 2006), and may lead to degrees of pollutant tolerance. In addition, the large number of intestinal lesions in the mice exposed to the high dose of POPs in our experiment may have contributed to a lower absorption rate of nutrients, which could have reduced the body weight of mice in this group.

Our findings of increased relative liver weights is in line with other studies where animals have been exposed to perfluorinated compounds (Seacat et al., 2003; Tan et al., 2013). These chemicals have been thoroughly investigated for hepatotoxicity, because of their high affinity to serum proteins and subsequent accumulation in the liver (Jones et al., 2003).

The highest concentration of POPs in our study is relatively large, but the low mixture concentration could potentially be considered more relevant for humans when taking life-long exposure and slow pollutant metabolism into account (Martignoni et al., 2006; Hudecova et al., 2018).

The occurrence of high mortality in offspring after neonatal AOM treatment (Experiment 2) was surprising, as the dosage used has not previously been associated with increased mortality. It is therefore unclear whether the lethality observed was caused by an abnormally high sensitivity to AOM, either alone or in combination with stress. However, we can conclude that the event was not caused by dietary POPs, since the AOM injection was given prior to weaning.

4.2. Effects of POPs on intestinal lesions

The process of cancer is divided into three phases; initiation, promotion and progression (Farber and Cameron, 1980). Depending on their mode of action, compounds may interfere with the molecular processes within each of these phases, and ultimately affect the carcinogenic process. Initiation is the irreversible heritable change in DNA, while promotion is the non-genotoxic advantages of mutated cell growth (Ludewig and Robertson, 2013). In the present study, the high mixture concentration of POPs initiated the formation of new lesions in the colon of A/J Min/+ mice, which was reflected by a significantly larger intestinal area covered by flat ACF. The low concentration of POPs did not affect the number of newly formed lesions, but promoted intestinal tumorigenesis by resulting in more colonic tumors of a larger diameter (>30 crypts). This initiating and promoting effect was not visible in the small intestine.

Previous studies have reported that some POPs affect both carcinogenic initiation and promotion *in vivo*. Liver tumorigenesis was initiated by a mixture of PCBs (Kanechlor 500) in mice (Ito et al., 1973). The same study also showed a promotional effect of the PCBs when administered together with hexachlorobenzene (HCB, α or β). Developmental exposure (*in utero* and via lactation) to dieldrin initiated the formation of mammary, ovarian and liver tumors in a transgenic mouse model for mammary tumorigenesis (Cameron and Foster, 2009). *In utero* exposure to perfluorooctanoic acid (PFOA) induced hepatocellular adenomas in CD-1 mice (Filgo et al., 2015). However, PFOA and PFOS did not increase the formation of intestinal lesions (Ngo et al., 2014). HCB was shown to promote mammary, liver and lung tumorigenesis in xenograft mouse models, without having initiating effects (Pontillo et al., 2013). The organochlorine metabolite *p,p'*-DDE has been suggested as a promoting agent in mammary tumorigenesis (Johnson et al., 2012). In addition, its parental compound *p,p'*-DDT (*p,p'*-dichlorodiphenyltrichloroethane) has been shown to promote CRC growth in mice injected with a suspension of the human colorectal adenocarcinoma cell line DLD1 (Song et al., 2014). The study also demonstrated that the CRC promotion by *p,p'*-DDT was achieved through the Wnt/β-catenin signaling pathway mediated by oxidative stress. *p,p'*-DDT elevated the production of reactive oxygen species (ROS), inhibited enzymes and reduced antioxidants levels in intestinal cells. Subsequently, there was an accumulation of β-catenin and the consecutive expression of target genes, which induced the proliferation of colorectal cancer cells and thus promoted CRC growth. The study also demonstrated that an increased production of ROS could affect colorectal carcinogenesis by interacting with specific pathways or by damaging DNA.

Furthermore, the metabolic activation of compounds may create products or intermediates that can interfere directly with DNA. PCBs have been shown to form highly reactive products and by-products that have the ability to mutate DNA, as reviewed by Ludewig and Robertson (2013). PBDEs are structurally similar to PCBs and have been shown to induce ROS formation, leading to chromosomal breakage (Ji et al., 2011). POPs may therefore have the ability to affect DNA and to increase tumorigenesis by inducing mutations in oncogenes or tumor suppressor genes such as *KRAS*, *p53* and *APC*. Changes in these genes are necessary for the development of colorectal cancer (Fodde, 2002). It has also been shown that most intestinal lesions in the Min/+ mouse have lost their remaining functioning *Apc* allele (Luongo et al., 1994). In the present study, mutations in *Apc* might have caused the formation of new lesions and enhanced the growth from flat ACF to tumors in the A/J Min/+ mice. However, this remains to be investigated.

4.3. Effects of AOM and POPs on intestinal lesions

AOM is converted to methylazoxymethanol (MAM) by cytochrome P450 enzymes (CYP450) located in both the liver and the intestines (Sohn et al., 2001). This highly reactive metabolite causes DNA mutations that are thought to initiate colorectal carcinogenesis (Takahashi and Wakabayashi, 2004). Different strains of mice vary in their susceptibility to AOM-induced CRC, and the A/J strain is known to be highly sensitive (Rosenberg et al., 2009). In addition, Min/+ mice exposed to AOM during their first two weeks of life have been shown to be particularly susceptible to induced and spontaneous intestinal carcinogenesis (Paulsen et al., 2003).

In the present study, neonatal mice in Experiment 2 were given one injection of AOM. This treatment did not seem to initiate the formation of new colonic lesions. Instead, it promoted the growth of already existing lesions, as evident from the increased number of tumors and colonic lesions of the larger size classes in mice from the control group. Combined exposure to AOM and POPs both

initiated and promoted colorectal carcinogenesis and resulted in a severe lesion burden, especially in mice exposed to the high mixture concentration of POPs. This large effect on tumorigenesis, compared to the relatively moderate initiation and promotion by POPs alone, indicates a synergistic effect between AOM and POPs. The high group exhibited the most extreme outcomes, which could be explained by the relatively high concentration of pollutants. However, the numerical differences from the control group demonstrate that the low mixture concentration also displayed initiating and promoting effects in the colon, as shown by a 7-fold increase in colonic tumor load. As with AOM, POPs are metabolized by CYP450 (Docea et al., 2017) and CYP450 has been shown to be a strong biomarker for the presence of POPs in animal tissue (Bachman et al., 2015). This similarity could be the origin of the synergistic effect observed between AOM and POPs, but this remains to be investigated. Previous studies in mice (Swiss and B6129SF2/J strains) have shown that PCBs promote carcinogenesis in lung and liver tissues when the tumors were initiated by N-nitrosodimethylamine (Anderson et al., 1994; Strathmann et al., 2006). The same promotional effect of PCBs was seen in A/J mice when given together with 1-Nitropropane to induce lung tumorigenesis (Nakanishi et al., 2001). The synergistic effect seen in the present study emphasizes the importance of anticipating synergistic effects between compounds which individually have the ability to initiate or promote cancer development. In addition, due concern should be given to chemical mixtures that not individually cause cancer, but which are disruptive in a manner that collectively provokes carcinogenesis (Goodson et al., 2015).

4.4. Histopathology

In the present study, the histopathological characterization of intestinal lesions differentiated between preneoplastic lesions, adenomas and carcinomas. The preneoplastic lesions included both hyperplastic and dysplastic cells. Dysplasia is a known hallmark of malignant potential and is closely related to *APC* mutations (Jen et al., 1994). Moderate to severe dysplasia has previously been shown in flat ACF from both traditional (C57BL/6) and A/J Min/+ mice (Paulsen and Alexander, 2001; Paulsen et al., 2006; Sødring et al., 2016a). In addition, flat ACF have been shown to be reliable surface biomarkers of *Apc*-driven colorectal carcinogenesis (Sødring et al., 2016a).

The initiating and promoting effect observed by intestinal scoring was not evident from the histopathological examination of mice only exposed to POPs (Experiment 1). However, carcinomas were observed in both the colon and small intestine, which could be an indication of the promotional effect explained above. In mice from Experiment 2, AOM and the high mixture concentration increased all types of lesion, which further confirms the synergistic effect observed between AOM and POPs. Interestingly, one injection of AOM alone did not cause the formation of colonic carcinomas. Instead, it induced the formation of preneoplastic lesions and adenomas, suggesting that AOM did not act as a tumor promotor in the A/J Min/+ mice after only one injection.

Because of the difference in method of analysis between intestinal scoring and histopathology, identical results could not be expected. However, similar trends were observed and both methods showed the same synergistic effect between AOM and POPs. It is important to emphasize that the histopathological examination was conducted on only three slides from each intestine. Thus, this method only investigates a small part of the area in question, compared to the scoring of the whole surface of the intestine, which could explain the discrepancy in findings between the two different methods.

5. Conclusion

The present study indicates that a mixture of POPs designed on the basis of human exposure, together with an injection of AOM, increased intestinal tumorigenesis in A/J Min/+ mice. Furthermore, a synergistic effect was observed between POP exposure and one injection of AOM. The results emphasize the importance of anticipating synergies when assessing the carcinogenic potential of compound mixtures.

Acknowledgements

The authors would like to thank the staff at Section for Experimental Biomedicine at The Norwegian University of Life Sciences for excellent care for the animals and support for the researchers. The study was funded by The Research Council of Norway (project name MultiPOP, project number 204361/H10).

References

Anderson, L.M., Logsdon, D., Ruskie, S., Fox, S.D., Issaq, H.J., Kovatch, R.M., Riggs, C.M., 1994. Promotion by polychlorinated biphenyls of lung and liver tumors in mice. Carcinogenesis 15, 2245–2248.

Bachman, M.J., Foltz, K.M., Lynch, J.M., West, K.L., Jensen, B.A., 2015. Using cytochrome P4501A1 expression in liver and blubber to understand effects of persistent organic pollutant exposure in stranded Pacific Island cetaceans. Environ. Toxicol. Chem. 34, 1989–1995.

Benhamouche, S., Decaens, T., Godard, C., Chambrey, R., Rickman, D.S., Moinard, C., Vasseur-Cognet, M., Kuo, C.J., Kahn, A., Perret, C., Colnot, S., 2006. Apc tumor suppressor gene is the "zonation-keeper" of mouse liver. Dev. Cell 10, 759–770.

Berntsen, H.F., Berg, V., Thomsen, C., Ropstad, E., Zimmer, K.E., 2017. The design of an environmentally relevant mixture of persistent organic pollutants for use in in vivo and in vitro studies. J. Toxicol. Environ. Health 80, 1002–1016.

Burt, R.W., 2000. Rare syndromes and genetic testing for colorectal cancer. Semin. Gastrointest. Dis. 11, 147–151.

Cameron, H.L., Foster, W.G., 2009. Developmental and lactational exposure to dieldrin alters mammary tumorigenesis in Her2/neu transgenic mice. PLoS One 4, e4303.

Colter, B.T., Garber, H.F., Fleming, S.M., Fowler, J.P., Harding, G.D., Hooven, M.K., Howes, A.A., Infante, S.K., Lang, A.L., MacDougall, M.C., Stegman, M., Taylor, K.R., Curran, C.P., 2018. Ahr and Cyp1a2 genotypes both affect susceptibility to motor deficits following gestational and lactational exposure to polychlorinated biphenyls. Neurotoxicology.

Darnerud, P.O., Atuma, S., Aune, M., Bjerselius, R., Glynn, A., Grawe, K.P., Becker, W., 2006. Dietary intake estimations of organohalogen contaminants (dioxins, PCB, PBDE and chlorinated pesticides, e.g. DDT) based on Swedish market basket data. Food Chem. Toxicol. 44, 1597–1606.

Docea, A.O., Vassilopoulou, L., Fragou, D., Arsene, A.L., Fenga, C., Kovatsi, L., Petrakis, D., Rakitskii, V.N., Nosyrev, A.E., Izotov, B.N., Golokhvast, K.S., Zakharenko, A.M., Vakis, A., Tsitsimpikou, C., Drakoulis, N., 2017. CYP polymorphisms and pathological conditions related to chronic exposure to organochlorine pesticides. Toxicol Rep 4, 335–341.

Farber, E., Cameron, R., 1980. The sequential analysis of cancer development. Adv. Canc. Res. 31, 125–226.

Filgo, A.J., Quist, E.M., Hoenerhoff, M.J., Brix, A.E., Kissling, G.E., Fenton, S.E., 2015. Perfluorooctanoic acid (PFOA)-induced liver lesions in two strains of mice following developmental exposures: PPARalpha is not required. Toxicol. Pathol. 43, 558–568.

Fodde, R., 2002. The APC gene in colorectal cancer. Eur. J. Canc. 38, 867–871.

Giannandrea, F., Gandini, L., Paoli, D., Turci, R., Figa-Talamanca, I., 2011. Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls. J. Environ. Sci. Health. B 46, 780–787.

Goodson 3rd, W.H., Lowe, L., Carpenter, D.O., Gilbertson, M., Manaf Ali, A., Lopez de Cerain Salsamendi, A., Lasfar, A., Carnero, A., Azqueta, A., Amedei, A., Charles, A.K., Collins, A.R., Ward, A., Salzberg, A.C., Colacci, A., Olsen, A.K., Berg, A., Barclay, B.J., Zhou, B.P., Blanco-Aparicio, C., Baglole, C.J., Dong, C., Mondello, C., Hsu, C.W., Naus, C.C., Yedjou, C., Curran, C.S., Laird, D.W., Koch, D.C., Carlin, D.J., Felsher, D.W., Roy, D., Brown, D.G., Ratovitski, E., Ryan, E.P., Corsini, E., Rojas, E., Moon, E.Y., Laconi, E., Marongiu, F., Al-Mulla, F., Chiaradonna, F., Darroudi, F., Martin, F.L., Van Schooten, F.J., Goldberg, G.S., Wagemaker, G., Nangami, G.N., Calaf, G.M., Williams, G., Wolf, G.T., Koppen, G., Brunborg, G., Lyerly, H.K., Krishnan, H., Ab Hamid, H., Yasaei, H., Sone, H., Kondoh, H., Salem, H.K., Hsu, H.Y., Park, H.H., Koturbash, I., Miousse, I.R., Scovassi, A.I., Klaunig, J.E., Vondracek, J., Raju, J., Roman, J., Wise Sr., J.P., Whitfield, J.R., Woodrick, J., Christopher, J.A., Ochieng, J., Martinez-Leal, J.F., Weisz, J., Kravchenko, J., Sun, J., Prudhomme, K.R., Narayanan, K.B., Cohen-Solal, K.A., Moorwood, K., Gonzalez, L., Soucek, L., Jian, L., D'Abronzo, L.S., Lin, L.T., Li, L., Gulliver, L., McCawley, L.J., Memeo, L., Vermeulen, L., Leyns, L., Zhang, L., Valverde, M., Khatami, M., Romano, M.F., Chapellier, M.,

Williams, M.A., Wade, M., Manjili, M.H., Lleonart, M.E., Xia, M., Gonzalez, M.J., Karamouzis, M.V., Kirsch-Volders, M., Vaccari, M., Kuemmerle, N.B., Singh, N., Cruickshanks, N., Kleinstreuer, N., van Larebeke, N., Ahmed, N., Ogunkua, O., Krishnakumar, P.K., Vadgama, P., Marignani, P.A., Ghosh, P.M., Ostrosky-Wegman, P., Thompson, P.A., Dent, P., Heneberg, P., Darbre, P., Sing Leung, P., Nangia-Makker, P., Cheng, Q.S., Robey, R.B., Al-Temaimi, R., Roy, R., Andrade-Vieira, R., Sinha, R.K., Mehta, R., Vento, R., Di Fiore, R., Ponce-Cusi, R., Dornetshuber-Fleiss, R., Nahta, R., Castellino, R.C., Palorini, R., Abd Hamid, R., Langie, S.A., Eltom, S.E., Brooks, S.A., Ryeom, S., Wise, S.S., Bay, S.N., Harris, S.A., Papagerakis, S., Romano, S., Pavanello, S., Eriksson, S., Forte, S., Casey, S.C., Luanpitpong, S., Lee, T.J., Otsuki, T., Chen, T., Massfelder, T., Sanderson, T., Guarnieri, T., Hultman, T., Dormoy, V., Odero-Marah, V., Sabbisetti, V., Maguer-Satta, V., Rathmell, W.K., Engstrom, W., Decker, W.K., Bisson, W.H., Rojanasakul, Y., Luqmani, Y., Chen, Z., Hu, Z., 2015. Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: the challenge ahead. Carcinogenesis 36 (Suppl. 1), S254–S296.

Howsam, M., Grimalt, J.O., Guino, E., Navarro, M., Marti-Rague, J., Peinado, M.A., Capella, G., Moreno, V., 2004. Organochlorine exposure and colorectal cancer risk. Environ. Health Perspect. 112, 1460–1466.

Hoyer, A.P., Jorgensen, T., Brock, J.W., Grandjean, P., 2000. Organochlorine exposure and breast cancer survival. J. Clin. Epidemiol. 53, 323–330.

Hudecova, A.M., Hansen, K.E.A., Mandal, S., Berntsen, H.F., Khezri, A., Bale, T.L., Fraser, T.W.K., Zimmer, K.E., Ropstad, E., 2018. A human exposure based mixture of persistent organic pollutants affects the stress response in female mice and their offspring. Chemosphere 197, 585–593.

IARC, 2016. Cancer Today. International Agency for Research on Cancer.

Ito, N., Nagasaki, H., Arai, M., Makiura, S., Sugihara, S., Hirao, K., 1973. Histopathologic studies on liver tumorigenesis induced in mice by technical polychlorinated biphenyls and its promoting effect on liver tumors induced by benzene hexachloride. J. Natl. Cancer Inst. 51, 1637–1646.

Jen, J., Powell, S.M., Papadopoulos, N., Smith, K.J., Hamilton, S.R., Vogelstein, B., Kinzler, K.W., 1994. Molecular determinants of dysplasia in colorectal lesions. Canc. Res. 54, 5523–5526.

Ji, K., Choi, K., Giesy, J.P., Musarrat, J., Takeda, S., 2011. Genotoxicity of several polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDEs, and their mechanisms of toxicity. Environ. Sci. Technol. 45, 5003–5008.

Johnson, N.A., Ho, A., Cline, J.M., Hughes, C.L., Foster, W.G., Davis, V.L., 2012. Accelerated mammary tumor onset in a HER2/Neu mouse model exposed to DDT metabolites locally delivered to the mammary gland. Environ. Health Perspect. 120, 1170–1176.

Jones, P.D., Hu, W., De Coen, W., Newsted, J.L., Giesy, J.P., 2003. Binding of perfluorinated fatty acids to serum proteins. Environ. Toxicol. Chem. 22, 2639–2649.

Kinzler, K.W., Vogelstein, B., 1996. Life (and death) in a malignant tumour. Nature 379, 19–20.

Kretzschmar, K., Clevers, H., 2017. Wnt/beta-catenin signaling in adult mammalian epithelial stem cells. Dev. Biol. 428, 273–282.

Ludewig, G., Robertson, L.W., 2013. Polychlorinated biphenyls (PCBs) as initiating agents in hepatocellular carcinoma. Canc. Lett. 334, 46–55.

Luongo, C., Moser, A.R., Gledhill, S., Dove, W.F., 1994. Loss of Apc+ in intestinal adenomas from Min mice. Canc. Res. 54, 5947–5952.

Martignoni, M., Groothuis, G.M., de Kanter, R., 2006. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expet Opin. Drug Metabol. Toxicol. 2, 875–894.

McGlynn, K.A., Quraishi, S.M., Graubard, B.I., Weber, J.P., Rubertone, M.V., Erickson, R.L., 2008. Persistent organochlorine pesticides and risk of testicular germ cell tumors. J. Natl. Cancer Inst. 100, 663–671.

Meunier, C., Kwan, T., Turbide, C., Beauchemin, N., Gros, P., 2011. Genetic control of susceptibility to carcinogen-induced colorectal cancer in mice: the Ccs3 and Ccs5 loci regulate different aspects of tumorigenesis. Cell Cycle 10, 1739–1749.

Mollersen, L., Paulsen, J.E., Alexander, J., 2004. Loss of heterozygosity and nonsense mutation in Apc in azoxymethane-induced colonic tumours in min mice. Anticancer Res. 24, 2595–2599.

Moser, A.R., Pitot, H.C., Dove, W.F., 1990. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 247, 322–324.

Nakanishi, Y., Bai, F., Inoue, K., Takayama, K., Pei, X.H., Harada, T., Izumi, M., Kimotsuki, K., Tokiwa, H., Hara, N., 2001. Polychlorinated biphenyls promote 1-nitropyrene-induced lung tumorigenesis without the induction of K-ras gene mutation in A/J mice. Teratog. Carcinog. Mutagen. 21, 395–403.

Nambiar, P.R., Girnun, G., Lillo, N.A., Guda, K., Whiteley, H.E., Rosenberg, D.W., 2003. Preliminary analysis of azoxymethane induced colon tumors in inbred mice commonly used as transgenic/knockout progenitors. Int. J. Oncol. 22, 145–150.

Ngo, H.T., Hetland, R.B., Sabaredzovic, A., Haug, L.S., Steffensen, I.L., 2014. In utero exposure to perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) did not increase body weight or intestinal tumorigenesis in multiple intestinal neoplasia (Min/+) mice. Environ. Res. 132, 251–263.

Paulsen, J.E., Alexander, J., 2001. Growth stimulation of intestinal tumours in Apc(Min/+) mice by dietary L-methionine supplementation. Anticancer Res. 21, 3281–3284.

Paulsen, J.E., Knutsen, H., Olstorn, H.B., Loberg, E.M., Alexander, J., 2006. Identification of flat dysplastic aberrant crypt foci in the colon of azoxymethane-treated A/J mice. Int. J. Canc. 118, 540–546.

Paulsen, J.E., Steffensen, I.L., Namork, E., Eide, T.J., Alexander, J., 2003. Age-dependent susceptibility to azoxymethane-induced and spontaneous tumorigenesis in the Min/+ mouse. Anticancer Res. 23, 259–265.

Pontillo, C.A., Rojas, P., Chiappini, F., Sequeira, G., Cocca, C., Crocci, M., Colombo, L., Lanari, C., Kleiman de Pisarev, D., Randi, A., 2013. Action of hexachlorobenzene on tumor growth and metastasis in different experimental models. Toxicol. Appl. Pharmacol. 268, 331–342.

Rosenberg, D.W., Giardina, C., Tanaka, T., 2009. Mouse models for the study of colon carcinogenesis. Carcinogenesis 30, 183–196.

Sancho, E., Batlle, E., Clevers, H., 2004. Signaling pathways in intestinal development and cancer. Annu. Rev. Cell Dev. Biol. 20, 695–723.

Seacat, A.M., Thomford, P.J., Hansen, K.J., Clemen, L.A., Eldridge, S.R., Elcombe, C.R., Butenhoff, J.L., 2003. Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. Toxicology 183, 117–131.

Sethi, S., Keil, K.P., Lein, P.J., 2017. Species and sex differences in the morphogenic response of primary rodent neurons to 3,3'-dichlorobiphenyl (PCB 11). Toxics 6.

Sohn, O.S., Fiala, E.S., Requeijo, S.P., Weisburger, J.H., Gonzalez, F.J., 2001. Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogens azoxymethane and methylazoxymethanol. Canc. Res. 61, 8435–8440.

Song, L., Zhao, J., Jin, X., Li, Z., Newton, I.P., Liu, W., Xiao, H., Zhao, M., 2014. The organochlorine p,p'-dichlorodiphenyltrichloroethane induces colorectal cancer growth through Wnt/beta-catenin signaling. Toxicol. Lett. 229, 284–291.

Strathmann, J., Schwarz, M., Tharappel, J.C., Glauert, H.P., Spear, B.T., Robertson, L.W., Appel, K.E., Buchmann, A., 2006. PCB 153, a non-dioxin-like tumor promoter, selects for beta-catenin (Catnb)-mutated mouse liver tumors. Toxicol. Sci. 93, 34–40.

Su, L.K., Kinzler, K.W., Vogelstein, B., Preisinger, A.C., Moser, A.R., Luongo, C., Gould, K.A., Dove, W.F., 1992. Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. Science 256, 668–670.

Sødring, M., Gunnes, G., Paulsen, J.E., 2016a. Detection and characterization of flat aberrant crypt foci (flat ACF) in the novel A/J Min/+ mouse. Anticancer Res. 36, 2745–2750.

Sødring, M., Gunnes, G., Paulsen, J.E., 2016b. Spontaneous initiation, promotion and progression of colorectal cancer in the novel A/J Min/+ mouse. Int. J. Canc. 138, 1936–1946.

Sødring, M., Oostindjer, M., Egelandsdal, B., Paulsen, J.E., 2015. Effects of hemin and nitrite on intestinal tumorigenesis in the A/J Min/+ mouse model. PLoS One 10, e0122880.

Takahashi, M., Wakabayashi, K., 2004. Gene mutations and altered gene expression in azoxymethane-induced colon carcinogenesis in rodents. Canc. Sci. 95, 475–480.

Tan, X., Xie, G., Sun, X., Li, Q., Zhong, W., Qiao, P., Sun, X., Jia, W., Zhou, Z., 2013. High fat diet feeding exaggerates perfluorooctanoic acid-induced liver injury in mice via modulating multiple metabolic pathways. PLoS One 8, e61409.

UNEP, 2015. Persistent Organic Pollutants (POPs).

Venning FA, C.M.a.K.H., 2013. The carcinogenic agent azoxymethane (AOM) enhances early inflammation-induced colon crypt pathology. J. Canc. Sci. Ther. 5 (11), 377–383.

Vestergren, R., Berger, U., Glynn, A., Cousins, I.T., 2012. Dietary exposure to perfluoroalkyl acids for the Swedish population in 1999, 2005 and 2010. Environ. Int. 49, 120–127.