

Original Article

Chloroacetanilide herbicide-induced rat enterochromaffin cell tumors: a case study within the context of the IPCS framework, for analyzing the relevance of a cancer mode of action for humans

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Abstract: The WHO International Programme on Chemical Safety (IPCS) framework for analyzing the relevance of a cancer mode of action (MoA) for humans (IPCS cancer-HRF) is an application to assess human relevance of tumorigenic hazards found through rodent bioassays. The chloroacetanilide herbicides, butachlor and alachlor, induced enterochromaffin-like (ECL) cell tumors in rat stomachs, at the highest doses. This study analyzed the human relevance of this tumor by applying the IPCS cancer-HRF using published data. In a postulated MoA, early key events (KEs) included decreased mucosal thickness in the fundic region, due to reduced parietal cells. The following KEs included increased pH of gastric acid and hypergastrinemia, leading to enhanced cell proliferation and hyperplasia, and resulting in the outcome of an ECL cell tumor. The data showed consistencies in dose-response and temporal concordance with the KEs and specificity in the tumor response, providing strengthened evidence of the KEs. While the early KE was not the same, similar MoAs have already been established for omeprazole and ciprofloxacin. The integrated data indicated that the postulated MoAs were biologically plausible. Alternative MoAs were excluded. Based on sufficient evidence, an MoA was established in rats. When addressing chemically inducible MoAs of human relevance, KEs of hypergastrinemia and trophic ECL cell hyperplasia were judged to not be qualitatively and quantitatively plausible in humans. The MoA in rats is unlikely to be present in humans; however, the potential effects on parietal cells cannot be excluded. Thus, the IPCS cancer-HRF is very useful for assessing human relevance. (DOI: 10.1293/tox.2021-0009; J Toxicol Pathol 2021; 34: 213–222)

Keywords: enterochromaffin-like cell tumors, chloroacetanilide herbicides, IPCS framework, human relevance, mode of action

Introduction

The chloroacetanilide herbicides, butachlor (IUPAC: *N*-butoxymethyl-2-chloro-2',6'-diethylacetanilide) and alachlor (IUPAC: 2-chloro-2',6'-diethyl-*N*-methoxymethylacetanilide), show chemical structure similarity, and are known to induce (at the highest dose tested) enterochromaffin-like (ECL) cell tumors in the glandular stomach in carcinogenicity studies on rats^{1,2} (Table 1). ECL cell tumor induction in rodent bioassays has not been reported for other chloroacetanilide herbicides^{3–5}. No genotoxicity has been reported for any chloroacetanilide herbicide, in an adequate battery of genotoxicity studies carried out *in vitro* and *in vivo*. Because ECL cell tumor induction by butachlor/

alachlor has been assessed as an effect of a non-genotoxic carcinogen, the acceptable daily intakes (ADIs) of both the compounds have been specified as 0.01 mg/kg body weight/day (bw/d) upon toxicological evaluation by the Food Safety Commission of Japan (FSCJ), the organization responsible for risk assessment of chemicals in foods in Japan.

To specify health-based guidance values for pesticide residues such as ADI, toxicity studies in compliance with Good Laboratory Practice (GLP) and in accordance with the guidelines of the Organization for Economic Cooperation and Development (OECD) or Ministry of Agriculture, Fishery and Forest (MAFF), have been requested. Recently, apart from these requested toxicity studies, mode of action (MoA) studies assessing the induced toxicity/carcinogenicity have also been conducted. MoA studies based on updated scientific knowledge are important for the interpretation of toxicity and/or carcinogenicity induced by a chemical. In particular, the consideration of an MoA of human relevance is crucial for dietary risk assessment of pesticide residues, because consumers have been taking in residue levels of pesticides through foods over the course of their entire lives.

Recently, the International Programme on Chemical Safety (IPCS) established a framework for analyzing the relevance of a cancer-/non-cancer MoA for humans (IPCS cancer-/non-cancer-HRF)^{6–8}. This consists of a series of key

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events (KEs) along the causal pathway to cancer, identified using a weight-of-evidence approach, based on the criteria of Sir Bradford Hill⁹. The Food and Agriculture Organization (FAO) and World Health Organization (WHO) Joint Meeting on Pesticide Residues (JMPR) has positively evaluated pesticide-induced cancer or non-cancer MoAs and their human relevance by applying the IPCS cancer-/non-cancer-HRF^{10–22}. Rodent liver tumor induction is the most popular example of applying the framework to toxicological evaluations of JMPR^{10–16, 21}. Recently, pydiflumetofen-induced liver tumors in male mice were assessed using the IPCS cancer-HRF²¹. The postulated MoA is constitutive androstane receptor-mediated liver tumor induction in mice. Various pieces of evidence on the KEs provided a high level of confidence that the postulated MoA was responsible for tumor outcomes in male mice. With regard to the human relevance of the postulated MoA, on the basis of the qualitative differences in the hepatocellular proliferation response to pydiflufenfen, it was established that this MoA is not relevant to humans²¹.

When the MoA of toxicity or tumor induced by a chemical is assessed using the IPCS cancer-/non-cancer-HRF, adequate data, including evidence from open literature, are needed to clarify the MoA. For butachlor and alachlor, a number of MoA studies on stomach tumors have been conducted by the applicants, and the summaries of these studies have been published in their risk assessment reports to FSCJ^{1, 2}. In addition, ECL cell tumors in rats are typical tumors for which MoA and human relevance have already been investigated in cases of gastric acid secretion inhibitors such as omeprazole or other classes of chemicals such as ciprofibrate^{23–25}. Therefore, it is predicted that adequate data are available for the assessment of the MoA of

butachlor-/alachlor-induced ECL cell tumors using the IPCS cancer-HRF.

The purpose of this analysis was to clarify the MoA of butachlor- and alachlor-induced ECL cell tumors, and to assess their human relevance by applying the IPCS cancer-HRF. All the data used in this study have been published. The author emphasizes that the views, thoughts, and opinions presented in this article are not necessarily those of FSCJ.

Materials and Methods

Materials

Two chloroacetanilide herbicides, butachlor and alachlor, were selected for the analysis because a number of MoA studies assessing the effects of these compounds on stomach tumor induction in rats have been conducted. The data analyzed included published reports for both the compounds. Major evidence were obtained from the risk assessment reports for both the compounds to FSCJ^{1, 2}, because the toxicological evaluations of both the compounds were conducted by toxicology experts using toxicity studies compliant with GLP and in accordance with guidelines authorized by the OECD or MAFF. Open literature written by the company's researchers^{26, 27} was also used as supportive information for the MoA.

Butachlor and alachlor have similar chemical structures and toxicological profiles^{1, 2}. The toxicological targets of both the compounds include the liver, nasal epithelium, glandular stomach, and thyroid. Liver, stomach, and thyroid tumors are also induced by both the compounds. Reproductive toxicity, teratogenicity, or genotoxicity relevant to humans has not been observed in case of either of the com-

Table 1. Histopathology of the Stomach in Chronic/Carcinogenicity Studies Carried out on Rats Treated with Butachlor and Alachlor

	Male				Female			
	0	100	1,000	3,000	0	100	1,000	3,000
Butachlor (SD rats)								
Concentration (ppm)	0	100	1,000	3,000	0	100	1,000	3,000
Dose (mg/kg bw/d)	0	4.5	45.6	139	0	5.7	58.5	190
No. of rats examined	78	80	80	79	80	78	80	80
Enterochromaffin cell hyperplasia (a)	0	0	0	1	0	0	0	4
ECL cell tumor, benign (a)	0	0	0	0	0	0	0	1
ECL cell tumor, malignant (a)	0	0	0	1	0	0	0	22*
Total incidence of ECL cell tumor	0	0	0	1	0	0	0	23*
Alachlor (Long-Evans rats)								
Dose (mg/kg bw/d)	0	14	42	126	0	14	42	126
No. of rats examined	49	50	50	50	50	50	50	49
Hyperplasia/gastritis	0	0	0	1	0	0	0	0
Enterochromaffin cell hyperplasia (a)	0	0	0	1	0	0	0	0
ECL cell tumor, malignant (a)	0	0	0	10*	0	0	0	20
Osteosarcoma	0	0	0	1 (b)	0	0	0	2 (b)
Mixed sarcoma	0	0	0	4 (b)	0	0	1 (b)	1 (b)
Total incidence in the glandular-stomach	0	0	0	15*	0	0	1	23*

(a) Diagnosis post peer review; (b) original diagnosis because peer review was not conducted; *Statistically significant difference from the control value at $p < 0.01$.

Reference sources^{1–2}.

pounds. The list of studies used in the current analysis of both the compounds is provided in Supplementary Table 1.

Methods

Analysis process using the IPCS cancer-HRF

The IPCS cancer-HRF has been described in several publications³⁻⁵. Briefly, the IPCS framework is mainly composed of two parts. The first part involves steps to check the sufficiency of the weight of evidence available to establish a MoA based on animal data, and the second part evaluates the human applicability of the proposed MoA found in animal experiments.

The first part has 9 steps to check causalities in animals, which are as follows:

- 1) Postulated MoA;
- 2) KEs and associated critical parameters;
- 3) Dose-response concordance;
- 4) Temporal association;
- 5) Strength, consistency, and specificity of the association of the tumor response with the KEs
- 6) Biological plausibility and coherence;
- 7) Possible alternative MoAs;
- 8) Uncertainties, inconsistencies, and data gaps;
- 9) Conclusion about the MoA.

The second part presents a four-step approach to address a series of three questions and leads to a documented and logical conclusion regarding the human relevance of the MoA underlying animal tumors. The four-step approach is as follows:

- 1) Is the weight of evidence sufficient to establish an MoA in animals?
- 2) Can the human relevance of the MoA be reasonably excluded on the basis of fundamental, qualitative differences in the KEs between the experimental animals and humans?
- 3) Can the human relevance of the MoA be reasonably excluded on the basis of quantitative differences in either the kinetics or dynamic factors between the experimental animals and humans?
- 4) Conclusion: statement of confidence, analysis, and implications.

Results and Discussion

Postulated mode of action and key events

Based on the integrated evidence of the MoA published in the risk assessment reports and the data from several open literature on butachlor and alachlor, an MoA of butachlor- and alachlor-mediated ECL cell tumor induction was postulated, as given below^{1, 2, 26, 27}:

The initial KE is that high-dose treatment with alachlor/butachlor causes a marked decrease in the parietal cells of rat glandular stomachs^{1, 2, 27}. Post absorption, butachlor/alachlor reduce the parietal cells in the fundic region of the glandular stomach. The reduced number of parietal cells leads to decreased mucosal thickness of the fundic region and mucosal atrophy. The prolonged reduction of

parietal cells causes consistent inhibition of acid secretion, which increases the pH of gastric acid. Subsequently, hyperplasia of antral cells and marked hypergastrinemia are observed. Hypergastrinemia stimulates enterochromaffin cell proliferation in the fundus, leading to ECL cell hyperplasia. The final outcome is ECL cell tumor formation. The postulated MoA is shown in Fig. 1.

KEs in the postulated MoA are as follows:

- A reduced number of parietal cells in the fundic region of the glandular stomach leads to decreased thickness of the fundic region and mucosal atrophy;
- Increased pH of gastric acid, due to inhibition of acid secretion;
- Hypergastrinemia;
- Increased activity of enterochromaffin cell proliferation;
- ECL cell hyperplasia.

Relevant data for the assessment of the MoA hypothesis for stomach tumors induced by butachlor and alachlor

Available data for the assessment of the IPCS-HRF analysis listed in Supplementary Table 1 were considered adequate, because information on the effect of dose-response and duration of treatment on the KEs/outcome could be accessed through the data.

Concordance of dose-response

The relationship between the KEs of ECL cell tumors and inducible doses of KEs was analyzed (Table 2). Based on the data in Tables 1 and 2, ECL cell tumors were observed for males and females at 139 and 190 mg/kg bw/d butachlor, respectively; effects were observed with 126 mg/kg bw/d alachlor for both the sexes in the carcinogenicity studies. These doses were the highest, indicating that the carcinogenic doses of both the compounds were over 100

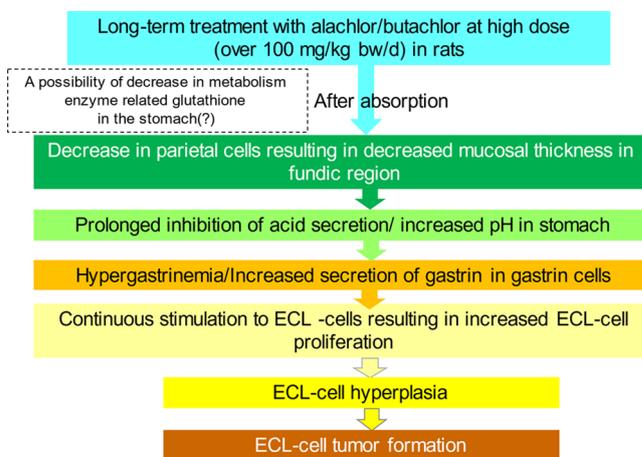


Fig. 1. Postulated MoA: ECL cell tumor induced by alachlor/butachlor and KEs. KEs, key events; MoA, mode of action; ECL cell, enterochromaffin-like cell; mg/kg bw/d, mg/kg body weight per day.

mg/kg bw/d.

Most KEs were observed at the same doses as the carcinogenic doses in case of both the compounds, except the reduction in the parietal cell number, decreased mucosal thickness, and mucosal atrophy. These early KEs were induced at lower doses than the carcinogenic dose, which was the highest dose tested for both the compounds. The dose-response results were considered reasonably concordant, because the early events cannot occur at doses higher than the carcinogenic dose.

Temporal association

The temporal concordance for the events associated with the fundic changes, such as decreased mucosal thickness or reduced number of parietal cells, is predicted to occur early following exposure to butachlor/alachlor after their absorption. A summary of the temporal associations is presented in Table 3.

The available data for each compound was not considered sufficient for the assessment of temporal concordance. However, a number of similarities, such as toxicological profiles, chemical structure, and postulated MoA, are known for butachlor and alachlor^{1, 2}. Therefore, the data for both the compounds were integrated to assess temporal associations. As a result, the earliest KE found was decreased mucosal thickness of the fundic region, which was observed 30 d post commencement of treatment. This change was consistent for 2 yr. Decreased gastric acid production and increased plasma/serum gastrin levels were found at 1 yr

(alachlor) and 180 d (butachlor/alachlor), although no measurements were conducted before these timings. Increased proliferation activity of ECL cells, a gastrin tropic event, was observed at 60 d (butachlor) and 120 d (alachlor), which was not observed until 30 d (butachlor) and 60 d (alachlor). ECL cell hyperplasia was detected at 1 yr, indicating late KE in the MoA. ECL cell tumor formation was initiated at 1 yr in case of alachlor and at 2 yr in case of butachlor. Overall, an outline of the temporal association can be drawn from the integrated data of both the compounds.

Strength, consistency, and specificity of the association of tumor response with KEs

The associations between tumor response and KEs are summarized in Table 4. For butachlor and alachlor, all the KEs were observed at the same or lower doses as those of ECL cell tumor formation, the outcome of the MoA. The evidence on dose-response indicated consistency of the association of the tumor response with the KEs. In the analysis of temporal concordance, KEs occurred in chronological order. Early KEs, such as decreased thickness of the fundic region due to a reduction in parietal cell number, were consistently found to occur earlier than the other KEs, such as increased pH of gastric acid and increased serum gastrin level, resulting in increased ECL cell proliferation and ECL cell hyperplasia. ECL cell tumors were formed after 1 yr of treatment with both the compounds. Recovery from ECL cell proliferation was observed in case of butachlor. The results of dose-response and temporal concordance

Table 2. Summary of the dose-response Relationship of KEs in ECL Cell Tumor Induction

		Dose levels (mg/kg bw/d)		
Butachlor		4.5–6.64	45.5–66.1	139–213
	Dose range in which a KE-related change was observed			
KE	Decreased parietal cell number	NE	Yes, but not significant	Yes
KE	Decreased mucosal thickness	No	No	Yes
KE	Mucosal atrophy	NE	Yes	Yes
KE	Increased pH of gastric acid	No	No	Yes
KE	Decreased gastric acid production	No	Increasing trend	Yes
KE	Increased serum gastrin	NE	No	Yes
KE	Increased activity of cell proliferation of enterochromaffin cells	NE	Yes	Yes
KE	ECL cell hyperplasia	No	No	Yes
Outcome	ECL cell tumor induction	No	No	Yes
Alachlor		14	42	126
KE	Decreased parietal cell number	NE	NE	Yes
KE	Decreased mucosal thickness	No	No	NE
KE	Mucosal atrophy	NE	Yes	Yes
KE	Decreased gastric acid production	NE	NE	NE
KE	Increased serum gastrin	NE	No	NE
KE	Increased activity of cell proliferation of enterochromaffin cells	NE	No	Yes (a)
KE	ECL cell hyperplasia	No	No	Yes
Outcome	ECL cell tumor induction	No	No	Yes

KE: key event; NE: not examined at these doses; mg/kg bw/d: mg/kg body weight per day; (a) increased cell proliferation activity was also observed at the dose of 252 mg/kg bw/d in females.

Reference sources^{1–2, 26–27}.

were reproducible in studies conducted using different experimental designs (Supplementary Table 1). The consistent temporal concordance in case of alachlor was not clearer than that in case of butachlor; however, integrated data from both the compounds indicated consistency of temporal concordance. Taken together, evidence on the consistency of dose-response and temporal concordance, as confirmed by multiple studies, supports the strong association of KEs with ECL cell tumors.

There was clear evidence of the specificity of ECL cell tumor formation in rats (Table 5). Butachlor or alachlor did not show any associated effects on ECL cell tumor formation in mice and dogs. A strain difference was also reported in rats; because of the decreased mucosal thickness of the fundic region, ECL cell proliferation of the glandular stomach or ECL cell tumor induction was not found in Fischer rats (Supplementary Table 1).

Biological plausibility and coherence

A comparison of the KEs between butachlor/alachlor and other compounds is summarized in Table 5. ECL cell tumors in rats are induced by other chemicals, such as omeprazole, a proton pump inhibitor, and ciprofloxacin, a potent lipidemic compound. ECL cell tumor induction by both the compounds was caused by the same MoA (Table 5): the early KE was a thickening of mucosa in the fundic stomach by blocking gastric acid secretion in the parietal cells. The following KEs included an increase in the pH of gastric acid, increased serum gastrin levels, and increased ECL cell

proliferation activity, resulting in ECL cell hyperplasia and the final outcome of ECL cell tumors^{23–25, 28}. Although the early KE of the omeprazole/ciprofibrate MoA was different from that of butachlor/alachlor, the consequent KEs and the final outcome were common between omeprazole/ciprofibrate and butachlor/alachlor.

The species specificity for omeprazole/ciprofloxacin-induced ECL cell tumor formation is also known. There is no definitive result that provides a reason for the species sensitivity in rats, but the anatomical or physiological specificity of the rat stomachs might be involved. In rats, ECL cell distribution in the fundic region of the glandular stomach is dense, and normal serum/plasma gastrin levels are high^{24–26}. Therefore, the postulated MoA and its KEs observed in a number of studies on both the compounds are considered biologically plausible and coherent.

Alternative MoAs

One alternative, MoA, is genotoxicity, including DNA reactivity. However, butachlor and alachlor showed no genotoxicity, in an adequate number of genotoxicity tests carried out *in vitro* and *in vivo*^{1, 2}. In addition, in the initiation-promotion assays of both the compounds, they showed no effect as initiators^{1, 2}. Therefore, a genotoxicity-based MoA was excluded from the analysis.

The next alternative MoA is direct damage to the stomach mucosa. If butachlor or alachlor has the potential to directly damage the stomach, any inflammatory changes that react to the damage, such as an erosion or ulceration,

Table 3. Summary of the Temporal Association of KEs in ECL Cell Tumor Induction

Finding	Duration						
	10–14 d	30 d	60 d	120 d	180 d	1 yr	2 yr
Butachlor							
KE	Decreased parietal cell number	NE	NE	NE	NE	Yes	NE
KE	Decreased mucosal thickness	No	Yes	Yes	Yes	Yes	Yes
KE	Mucosal atrophy	NE	NE	NE	NE	Yes	Yes
KE	Increased pH of gastric acid	NE	NE	NE	NE	NE	Yes
KE	Decreased gastric acid production	NE	NE	NE	NE	NE	Yes
KE	Increased serum gastrin	NE	NE	NE	Yes	Yes	Yes
KE	Increased activity of cell proliferation of enterochromaffin cells	No	No	Yes	Yes	Yes	Yes
KE	ECL cell hyperplasia	No	No	No	No	No	Yes
Outcome	ECL cell tumor induction	No	No	No	No	No	Yes
Alachlor							
KE	Decreased parietal cell number	NE	NE	NE	NE	NE	Yes
KE	Decreased mucosal thickness	NE	NE	NE	NE	Yes	NE
KE	Mucosal atrophy	NE	NE	NE	NE	Yes	NE
KE	Increased pH of gastric acid	NE	NE	NE	NE	Yes	NE
KE	Decreased gastric acid production	NE	NE	NE	NE	Yes	NE
KE	Increased serum gastrin	NE	NE	NE	Yes	NE	NE
KE	Increased activity of cell proliferation of enterochromaffin cells	No (a)	NE	No (a)	Yes	NE	NE
KE	ECL cell hyperplasia	No	No	No	No	No	Yes
Outcome	ECL cell tumor induction	No	No	No	No	Yes	Yes

KE: key event; d: days; yr: year(s); NE: not examined; ECL cell: enterochromaffin-like cell; (a) the cell proliferation activity increased at the dose of 252 mg/kg bw/d, which was higher than 126 mg/kg bw/d, the carcinogenic dose, from 10 days onwards. At 126 mg/kg bw/d, the carcinogenic dose, the increased was observed from oed weight per day from 120 days onwards.

Reference sources^{1–2, 27}.

Table 4. Evidence to Show the Strength, Consistency, and Specificity of the Association of Tumor Response with KEs

Chronological order of KEs in the postulated MoA	Evidence	The lowest dose observed (mg/kg bw/d)	When was the effect observed?	No. of studies in which reproducibility was found
Butachlor				
Early KE	Decreased mucosal thickness	213	From 30 d	5
	Decreased parietal cell number	66	12 months	
	Mucosal atrophy	139	From 1 yr	
Post mucosal change	Decreased gastric acid production	66.1	1 yr	2
	Increased pH of gastric acid	213	2 yr	
Post decreased gastric acid production	Increased serum gastrin	213	From 180 d	1
Post hypergastrinemia	Increased activity of ECL cell proliferation	213	From 60 d	2
Post prolonged cell proliferation	ECL cell hyperplasia	213	2 yr	1
Outcome	ECL cell tumor induction	213	2 yr	2
Did the evidence show recovery?		213	60 d	1
Is the dose consistently the same as or lower than the carcinogenic dose?		Yes		
Did the KEs occur consistently and in chronological order?			Yes	
Did the evidence show reproducibility?				Yes
Alachlor				
Early KE	Decreased mucosal thickness	126	From 1 yr	3
	Decreased parietal cell number	126	2 yr	
	Mucosal atrophy	126	From 1 yr	
Post mucosal change	Increased pH of gastric acid	126	1 yr	1
Post decreased gastric acid production	Increased serum gastrin	126	From 180 d	1
Post hypergastrinemia	Increased activity of ECL cell proliferation	126	From 120 d	1
Post prolonged cell proliferation	ECL cell hyperplasia	126	2 yr	1
Outcome	ECL cell tumor induction	126	From 1 yr	2
Is the dose consistently the same as or lower than the carcinogenic dose?		Yes		
Did the KEs occur consistently and in chronological order?			Not clear due to only one measurement	
Did the evidence show reproducibility?				Yes, but some

KE: key event; MoA: mode of action; mg/kg bw/d: mg/kg bodyweight per day; ECL cell: enterochromaffin-like cell; d: days; yr: year(s). Reference sources^{1-2, 26-27}.

should have been induced in the surface epithelium of the stomach. However, no inflammatory changes were observed for either compound^{1, 2}. In addition, radiolabels of both the compounds were not found to be distributed in the stomach during the absorption, distribution, metabolism and excretion (ADME) studies^{1, 2, 27}. Therefore, this MoA was also excluded.

Toxic metabolite formation in the stomach should also be considered, because butachlor and alachlor are known to induce species-specific nasal tumors via toxic metabolite formation in the nasal cavity. In the ADME studies, radiolabels of both the compounds were not found to be distributed in the stomach. Therefore, this MoA was also excluded^{1, 2, 27}.

ECL cell tumors are known to be induced in rats by medical drugs such as omeprazole, to inhibit gastric acid secretion, as described above. The early KE of this MoA was morphologically characterized as a thickened fundic region²⁹. Although the other KEs of these blocking enzymes were similar to those of butachlor and alachlor, the early KE was different²⁸.

Uncertainties, inconsistencies, and data gaps

The most important uncertainty is that the initial molecular KE that induces parietal cell number reduction remained undetermined in case of butachlor and alachlor. This KE was different from that of omeprazole and ciprofloxacin. There have been several studies on glutathione measurement in the liver or stomach with butachlor/alachlor; however, no clear results were obtained to provide evidence that glutathione depletion occurs in the fundic region or active metabolite formation (Supplementary Table 1). The MoA of the initial KE leading to reduced parietal cell counts/decreased mucosal thickness of the fundic region should be clarified in rats. If there is no evidence showing the MoA, these effects observed as initial KE and leading KE should be identified as the effects relevant to humans.

Data gaps in temporal association existed because of the inappropriate timing of measurements of KEs. The chronological occurrence of alachlor was not clear compared to that of butachlor. However, as described in the temporal association, the outline of the temporal association could be drawn from the integrated data of both the compounds. No data inconsistency was observed.

Table 5. Species Comparison of Gastric Changes Induced by Butachlor/Alachlor and Other Chemicals during ECL Cell Tumor Induction in Rats, as well as, in the Anatomy/Physiology of the Stomach

Evidence	Rat	Mouse	Dog	Monkey/ marmoset	Human
Butachlor^{1-2, 27a}					
Decreased mucosal thickness	Yes	No	No	No	
Increased pH of gastric acid	Yes	NE	NE	NE	
Increased serum/plasma gastrin level	Yes	NE	NE	NE	
ECL cell proliferation	Yes	No	No	No	
ECL cell hyperplasia	Yes	No	No	NE	
ECL cell tumor	Yes	No	No	NE	
Omeprazole^{23, 28a}					
Gastric mucosa thickening	Yes	NE	Yes*	NE	
Increased serum/plasma gastrin level	Yes, marked	NE	NE	NE	
ECL cell hyperplasia	Yes	NE	Yes*	NE	
ECL cell tumor	Yes	No	No	NE	
Ciprofibrate^{24-25a}					
Gastric mucosa change	Yes	No	NE	No	
Increased serum/plasma gastrin level	Yes	No	No	No	No
Morphological change in ECL cells	Yes	No	NE	No	
ECL cell hyperplasia	Yes	No	NE	No	
ECL cell tumor	Yes	No	NE	NE	
Anatomical/physiological profiles					
ECL cell density ^{23, 26a}	High	NE	NE	NE	Low
Normal serum gastrin level (pg/mL) ^{24-26, 31}	170-360	70-110	NE	47-90	40-180
Serum gastrin level of patient with gastrinoma (pg/mL) ³¹					<1,000
Serum gastrin level of patient with atrophy gastritis (pg/mL) ³¹					Increase, <1,000

ECL cell: enterochromaffin-like cell; ^a, data obtained from reference; *, This effect was observed at a 10-fold higher range than the same effective dose in rats. NE: not examined.

Assessment of the postulated MoA

The data provide adequate support for a non-genotoxic threshold-based MoA for the development of ECL cell tumors in rats with chronic exposure to butachlor or alachlor^{1, 2}. The KEs for the MoA were identified and supported by consistent data on dose-response and temporal concordance. In addition, this MoA is similar to the MoA of ECL cell tumor induction by omeprazole and ciprofloxacin in rats, except for the difference in the early KE. Taken together, the postulated MoA of ECL cell tumor induction was strongly supported by consistent evidence of KE associated with the tumor. 3.10. Human relevance of the postulated MoA Section 3.10 describes Step 2 of the IPCS cancer-HRF.

Is the weight of evidence sufficient to establish the MoA in animals?

Yes, the toxicological data support a non-genotoxic, threshold-based MoA for the development of ECL cell tumors in the glandular stomach of rats post chronic and high-dose exposure to butachlor or alachlor. The weight of evidence supports an increased pH of gastric acid and increased gastrin-tropic continuous ECL cell proliferation activity. The early KE is considered a different MoA from the known MOAs of omeprazole and ciprofloxacin in rats.

Can the human relevance of the MoA be reasonably excluded on the basis of fundamental/qualitative differences in key events between humans and animals?

Qualitatively, continuously higher plasma/serum gastrin levels may pose a potential risk of ECL cell tumors in the stomach of humans, because hypergastrinemia is considered to induce ECL cell hyperplasia in patients with gastrinoma or chronic atrophic gastritis³⁰. In humans, the increased levels of serum gastrin are higher in patients with gastrinomas than in those with atrophic gastritis³¹. In addition, carcinoid tumors or gastrinomas have rarely been detected in humans. Even when hypergastrinemia is marked and prolonged, such as in patients with Zollinger-Ellison syndrome, gastric carcinoids are rare²⁸. When considering the inducible possibility of hypergastrinoma leading to increased activity of ECL cells by any treatment, it is widely understood to be an effect of exaggerated pharmacodynamic activity not directly relevant to the therapeutic dose of these drugs, such as omeprazole²⁸. KEs were not observed in animals other than those treated with omeprazole or ciprofloxacin (Table 6). Anatomical and physiological specificities exist in the stomach of rats. Therefore, MoA in rats is unlikely to lead to ECL cell tumors in humans at levels of exposure arising from pesticide residues in food.

Can the human relevance of the MoA be reasonably excluded on the basis of quantitative differences in KEs between the experimental animals and humans?

Yes. As mentioned above, the human relevance of the MoA can reasonably be excluded based on qualitative differences in the toxicology data. Margins between inducible doses of the ECL cell tumor (over 100 mg/kg bw/d for both the compounds) and an ADI of 0.01 mg/kg bw/d for both the compounds, which are health-based guidance values for chronic exposure, were over 10,000^{1, 2}. Consumers are not predicted to be exposed to butachlor/alachlor at such dose levels via food.

Statement of confidence, analysis, and implications of IPCS cancer-HRF

There is sufficient evidence to establish the ECL cell tumor MoA for butachlor/alachlor in rats. There are no human data on chronic exposure to butachlor/alachlor, and there is no evidence on chemicals with the same MoA as

butachlor/alachlor-induced gastrin-tropic ECL cell proliferation in humans, as the early KEs were not the same. When IPCS cancer-HRF was applied to ECL cell tumor induction by butachlor and alachlor, the MOA established in rats was reasonably excluded from human relevance, both qualitatively and quantitatively. Based on the MoA, ECL cell induction in rats was found to be irrelevant to humans. Each step in the assessment of the postulated MoA in rats for human relevance is shown in Fig. 2.

Conclusion of the current analysis and applicability of IPCS cancer-HRF

The current study indicated that the MoA of ECL cell tumors induction by butachlor and alachlor and its relevance to humans could be analyzed using the IPCS cancer-HRF. The MoA of ECL cell tumors induced in rats is not considered to be extrapolatable to humans. The hazard potential that the exposure to these compounds induces a change in the parietal cell number (an early KE) in humans cannot be

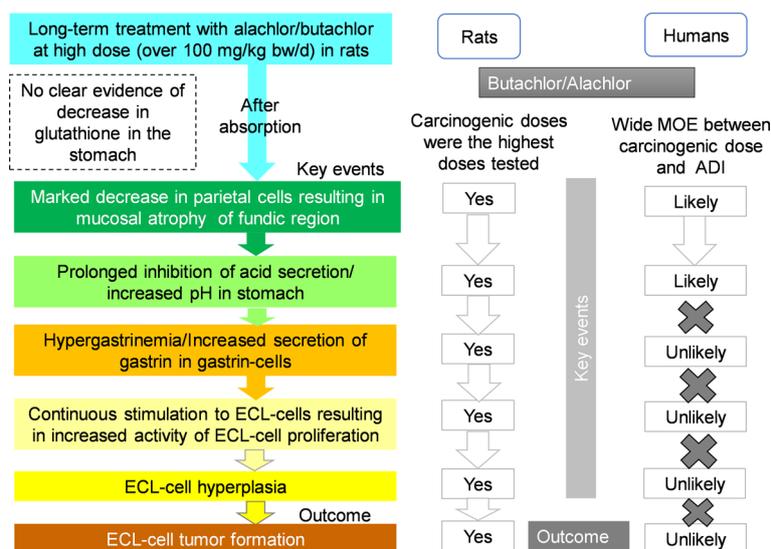


Fig. 2. Human relevance of MoA in butachlor-/alachlor-induced ECL cell tumor induction. MoA, mode of action; ECL cell, enterochromaffin-like cell; mg/kg bw/d, mg/kg body weight per day.

Table 6. Human Relevance of KEs in the Postulated MoA of ECL Cell Tumor Induction by Butachlor and Alachlor

KE-related change	Rat	Human	Comments
KE Decreased parietal cell number	Yes	Likely	If gastric acid secretion is reduced in parietal cells, any reaction may occur in the fundic mucosa as a hazard. The reactive change was species-specific for omeprazole or ciprofibrate.
KE Decreased mucosal thickness/mucosal atrophy	Yes	Likely	
KE Increased pH of gastric acid and related findings	Yes	Likely	Increased pH of gastric acid is predictable because omeprazole has been used for increasing the gastric acid pH in humans.
KE Increased serum gastrin	Yes	Unlikely	No evidence of changing gastric secretion, except in rats.
KE Increased activity of proliferation of ECL cells	Yes	Unlikely	Omeprazole-mediated ECL cell hyperplasia has been reported in dogs, at a 10-fold higher dose than that in rats. No evidence of chemically-induced ECL cell proliferation/hyperplasia, except in rats.
KE ECL cell hyperplasia	Yes	Unlikely	No evidence of chemically-induced ECL cell tumors, except in rats.
Outcome ECL cell tumor induction	Yes	Unlikely	No evidence of chemically-induced ECL cell tumors, except in rats.

KE: key event, MoA: mode of action; ECL cell: enterochromaffin-like cell.

excluded, due to insufficient evidence to clarify the early KE.

An adverse outcome pathway (AOP) is known as a logical sequence of causally linked events at different levels of biological organization, which follows exposure to a chemical and leads to adverse health effects in humans³². The IPCS cancer-/non-cancer-HRF is more specific to toxicity or carcinogenicity induced by a target chemical than AOP, because the outcome results from the target chemical treatment, and results of toxicological or mechanistic studies of the target chemical are used in each analytical step of the postulated MoA. Compound-specific analysis using the IPCS cancer-/non-cancer-HRF is considered suitable for toxicological assessments of MoA and human relevance conducted by regulatory organizations. In conclusion, the IPCS cancer-HRF is a very effective tool for assessing the human relevance of cancers induced by chemicals.

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Disclaimer: The author states that the views, thoughts, and opinions presented here are not necessarily those of FSCJ.

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