Antigestational effects of *Icon*®, a pyrethroid insecticide on mid pregnancy of rats

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Abstract

Icon® is a water miscible type II synthetic pyrethroid insecticide based on active ingredient lambda cyhalothrin (10% w/v). It was recently introduced to Sri Lanka as an indoor spray against malaria vector mosquitoes. The aim of this study was to ascertain the potential effects of *Icon*® on pregnancy outcome of rats when exposed during mid pregnancy (days 8 –14). *Icon*® was orally administered daily during this period in three different doses: 63, 83, 125 mg/kg body wt./day of *Icon*® (active ingredient; lambda cyhalothrin ; 6.3, 8.3, 12.5 mg/kg/day) respectively. Several parameters of reproduction, pre- and post-natal development were monitored. The results demonstrated that *Icon*® is detrimental to pregnancy outcome (in terms of number of uterine implants, number of viable implants, post implantation loss, number of pups born, litter index, and foetal survival ratio) but induced no changes in gestational length, gross morphological birth abnormalities or detectable pre- and post- natal developmental impairments. The anti-reproductive effects of *Icon*® were mediated by multiple mechanisms (hypophagia, maternal toxicity, stress, uterine myotropic activity, embryo-foetoxicity, anti-progestrogenic activity, inhibition of decidualizaton process, abortifacient activity and vaginal bleeding) leading to enhancement of post–implantation losses. It is concluded that exposure to *Icon*® during mid gestation poses a considerable threat to pregnancy of rats.

Key words : *Icon*®, Insecticide, lambda cyhalothrin, pyrethroids, pregnancy, mid gestation, post-implantation loss, reproduction

Introduction

Icon®, produced by the Public Health Division of the Imperial Chemical Industries in England (currently known as Zeneca) (1) is a water miscible type II synthetic pyrethroid insecticide having lambda - cyhalothrin (10% w/v) as the active ingredient (2). Information on inert fillers, adjuvants, excipient, wetting agents and purity are not available. This insecticide was recently introduced to Sri Lanka to use as an indoor spray against malaria vector mosquitoes. *Icon*® is a contact and residual insecticide and acts as a neuropoison by interfering with the conductance of nerve

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membrane by prolonging the sodium current (2, 3). Pyrethroids are also known to increase spontaneous release of neurotransmitters such as GABA, dopamine and noradrenaline, and also act as a hormone disruptor (4, 5).

The effective spray dose of *Icon*® in Sri Lanka is 312 mg/m^2 (CIC brochure and personal communication K. Gunasekara, Parasitologist, Anti Malaria Campaign, Sri Lanka,). Insecticide residues in indoor environments are more persistent than in the environment at large, as indoors residues are not subjected to degradation by sun, rain and soil microbes and hence there is a potential risk that inhabitants in *Icon*®-sprayed houses are continually subjected to dietary, respiratory and cutaneuos exposures (2).

Recently we showed that *Icon*® exposure to male rats impaired sexual competence (6) and in female rats when exposed during early gestation was detrimental to their pregnancy outcome (7). Several pesticides when exposed during mid pregnancy are known to interrupt pregnancy (8, 9, 10, 11, and 12). However, the potential anti-reproductive effects of *Icon*® when exposed during mid pregnancy is not known and this is worth examining as *Icon*® is an endocrine disruptor (7).

The aim of this study was to investigate the effects of *Icon*® on reproductive outcome of female rats subjected to repeated exposure during mid pregnancy. This was done using doses of *Icon*® (approximately 2.5 to 4 times lower than the recommended spray dose in Sri Lanka) identical to what has been used to ascertain reproductive effects when exposed during early pregnancy (7).

Material and Methods

Animals

Healthy adult cross bred female rats (weight: 200–250 g) and male rats (weight: 200–250 g) of proven fertility from the colony maintained at the Department of Zoology, University of Colombo were used. They were maintained singly in plastic cages under standardized animal house conditions (temperature: 28–30 °C; photoperiod: approximately 12 h light/12 h dark; and relative humidity: 50–55 %) with free access to pelleted food (Master Feeds Lanka Ltd., Colombo, Sri Lanka) and tap water. Except at the time of experiment the animals were handled only during cage cleaning.

Icon® preparation

Icon® was obtained from Anti-Malaria Campaign, Narahenpitiya, Colombo 5, Sri Lanka. Three doses [63 (low), 83 (mid) and 125 (high) mg/kg/day; (containing active ingredient lambda cyhalothrin 6.3, 8.3, 12.5 mg /kg/day respectively)] of *Icon*® in 1ml aliquots were prepared by mixing *Icon*® in distilled water (DW). These doses are comparable to what has been used previously by us to investigate the potential reproductive effects on male rats (6) and identical to what has been used to test the anti-reproductive effects on female rats when exposed during early pregnancy (7).

Icon® administration

Icon® or vehicle (DW) was orally administered by gastric intubation (09.00 – 10.00 h) from days 8-14 of pregnancy. The rats were made pregnant by individually pairing pro-oestrous females overnight with a male rat. Vaginal smears were examined on the following morning (08.00–09.00h)

for presence of spermatozoa (considered as day 1 of pregnancy).

Adverse effects

Following every dosing, cage side observations were made on each rat continuously for 3–5 h for mortality, overt signs of toxicity (salivation, lacrymation, ptosis, squinted eyes, wilting, tremors, convulsions, ataxia, yellowing and loss of fur), stress (exophthalmia, erection of fur), lethargy (reduction of spontaneous walking movements, climbing in cage, cleaning of fur) recumbence, aversive behaviours (self biting and scratching, licking of tail and/or paw, intense self grooming behaviour and vocalization), diarrhoea, colour and odour of urine and vaginal bleeding. In addition, the rectal temperature was recorded on day 8 of pregnancy before dosing and on day 14 of pregnancy (5 h after dosing) using a clinical thermometer (Oson Duopris Company Ltd., Berlin, Germany).

Effect on food and water intake

Food and water intake of *Icon®* treated rats [low (n = 12), mid (n = 12), high (n = 9)] or control (n =12) were determined daily from days 8-14 of treatment using conventional laboratory techniques (13).

Effect on body weight

The body weights of *Icon®* treated rats [low (n = 12), mid (n = 12), high (n = 9)] or control (n =12) were determined 5 h after dosing on days 8 and 14 of pregnancy using an electronic balance (MP6000, Chyo YMC & Corporation Ltd., Japan).

Effect on haematology

On day 1 post-treatment [low (n = 12), mid (n = 12), high (n = 9) or control (n =12)], blood was collected from tails under aseptic conditions and red blood cell (RBC) counts, white blood cell (WBC) counts, differential leukocyte (DC) counts, packed cell volume (PCV) and haemoglobin content were estimated as described by Cheesbrough *et. al.*, (14). The mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were then computed.

Evaluation of sedative potential

The sedative potential of *Icon®* was evaluated using the rat hole–board technique (15). Forty-five, day 8 pregnant rats were assigned into 4 groups and orally treated with *Icon®* [low (n = 12), mid (n = 12), high (n = 9)] or vehicle (n =12) for 7 consecutive days. Five hours after the last dosing, rats were individually placed on the centre of the rat hole-board and were given 7.5 min trial periods. During this period the number of head dips, rears and locomotary activity were noted.

Effect on righting reflex

The time for righting reflex on rats used in evaluating the sedative potential were determined 2 h prior to treatment and immediately after hole-board test as described by Mortin *et. al.*, (16).

Effect on pregnancy outcome and pre- and post-natal development of pups

Forty-five, day 8 pregnant rats were assigned into 4 groups and orally administered with either different doses of *Icon®* [low (n = 12), mid (n = 12), high (n = 12)] or vehicle (n =12) for 7 consecutive days.

On day 17 of pregnancy, these rats were subjected to a laporatomy under mild ether

anesthesia with aseptic precautions. Upon laporatomy, the total number of uterine implants, the number of viable implants (with pulsating blood vessels and reddish appearance), and the number of dead implants (with non pulsating blood vessels and bluish appearance) were recorded. The appearance and number of corpora lutea in ovaries were also noted. Furthermore, the distribution of embryos in the uterine horns, and the diameter of the first embryo at the ovarian end were measured using a vernier caliper (Gallenkamp, Loughborough, UK). Subsequently the laparatomy incision was sutured. Tetracycline ointment (Wockhardt Veterinary Ltd., Bombay, India) was applied and was injected with a subcutaneous dose of 0.1 ml tetracycline (Wockhardt Veterinary Ltd., Bombay, India). Then the animals were allowed to recover and deliver. The gestation length was recorded.

After delivery, on postnatal day 1, the pups were closely observed and the total number of pups, the total number of viable pups, their body weights (using an electronic balance), cranial length, cranial diameter, cranio-sacral length and tail length of each pup (using a vernier caliper) were determined. The presence of gross external congenital abnormalities were also noted (amelia, anomaly of tail, clubfoot, oligodactyly or syndactyly). Then the pups were observed once daily for the appearance of fur and opening of eyes. The following reproductive indices were computed using the reproductive findings.

Post-implantation loss (%) = [(# implants - # viable implants) / # implants x 100; Gestation index= (# live litter / # pregnant) x 100; Litter index (%) = (# littered pups / # implants) x 100; Live birth index (%) = (# viable pups / # littered pups) x 100; Foetal survival ratio (%) = (# surviving pups / # implants) x 100; Viability index (%) = (# day 1 surviving animals / # live pups per animal) x 100].

Statistical analysis

Data are expressed as means ± standard error of mean (SEM). Mann-Whitney *U*-test and G-test were used as appropriate. $P < 0.05$ was considered as statistically significant.

Results

Adverse effects

No deaths were recorded in any of the treated rats. Further, none of the treated rats showed, lacrymation, ptosis, squinted eyes, wilting, tremors, convulsions, yellowing and marked loss of fur, and aversive behaviours.

As shown in the Table 1, *Icon*® produced ataxia, exophthalmia, piloerection, salivation (low dose: around facial region; mid dose: neck region; and high dose: almost throughout the entire body), vaginal bleeding (between days 9–12 of pregnancy), diarrohea (between days 9–14 of pregnancy) and pale yellow (low dose) to dark yellow (mid dose and high dose) coloured urine with an odour similar to that of *Icon*® between days 8–14 of pregnancy. These toxic effects were evident from 30–60 min. of administration of *Icon*® and were completely abolished on the following morning except with the high dose where the effects lasted for 2 days after the full treatment course. *Icon*® administration did not significantly alter the rectal temperature of any treated rats ($P > 0.05$; Mann-Whitney *U*–test).

Effect on food and water intake

The highest dose of *Icon®* induced a significant impairment in food consumption from days 3–7 of treatment ($P<0.05$; Mann-Whitney *U*–test). In contrast, there was no significant inhibition of water intake in any of the *Icon®* treated rats [$P>0.05$; Mann-Whitney *U*–test, data not shown].

Effect on body weight

Body weight gain was significantly impaired by *Icon®*; low (by 60%), mid (by101%) and high (by 196%): weight gain of control, low, mid and high dose groups respectively being 12.9 ± 2.1, 5.1 ± 2.5, -0.1 ± 2.2 and –12.4 ± 4.1 g) ($P<0.05$; Mann-Whitney *U*–test).

Haematology

Of the haematological variables investigated both mid (by 39%) and high (by 72%) doses of *Icon®* significantly increased the WBC count (control vs. mid vs. high dose: 1.8 ± 0.1 vs. 2.5 ± 0.1 vs.3.1 ± 0.2 x $10^4/mm^3$). The highest dose also significantly increased the PCV (by 10%) (control vs. high dose: 40.6 ± 0.7 vs. 44.7 ± 0.7 %) and MCV (by 15%) (control vs. high dose: 44.6 ±1.7 vs. 51.4 ± 2.3) ($P<0.05$; Mann-Whitney *U*–test).

Sedative effects

None of the three doses of *Icon®* significantly impaired any of the variables investigated (data not shown) ($P>0.05$; Mann-Whitney *U*–test).

Righting reflex

Only the highest dose of *Icon®* significantly enhanced the righting reflex time ($P>0.05$; Mann-Whitney *U*–test) (data not shown).

Reproductive out come, pre- and post-natal development of pups

The results obtained are summarized in Tables 2 and 3. All treated rats had normal numbers of reddish, rounded, seemingly healthy looking corpora lutea. The low dose of *Icon®* when administered between days 8 –14 of pregnancy had no significant effect on any of the variables investigated. In contrast, the mid dose significantly impaired the number of uterine implants (by 28%), the number of viable implants (by 37%), the number of pups born (by 37%), the number of live pups born (by 40%), the litter index (by 44%), and the foetal survival ratio (by 34%). Further, these anti-reproductive effects were accompanied by a marked and significant increase of post–implantation losses (mid dose by 82% and high dose by 1512%). In contrast, with the high dose of *Icon®*, no uterine implants were seen with a 100% post–implantation loss. Because of this the other variables listed could not be investigated.

The pups born with both low and mid doses of *Icon®* appeared normal with no gross external abnormalities. None of the pre- and post-natal developmental variables investigated were significantly altered both by the low and mid doses of *Icon®* (Table 3).

Discussion

This study examined the anti-gestational effects of *Icon®* when exposed orally during mid pregnancy of rats (days 8 –14). The results demonstrated that, in rats, repeated oral exposure of *Icon®* during mid gestation is detrimental to pregnancy outcome (in terms of the number of uterine implants, number of viable implants, post

implantation loss, number of pups born, litter index and foetal survival ratio). Previous experiments have shown that exposure of rats to *Icon*® during early pregnancy does not produce any impairment in pre- and post-natal development of pups or gross birth defects (7). Still we cannot completely rule out any *Icon*®-induced minor and subtle effects on embryo/ feototoxicity and pup development. In contrast, both teratogenic and developmental retardation have been reported with some pyrethroid (9, 17, 18), carbamate (10, 11), organophosphate (12) and organochlorine (19) insecticides following mid pregnancy exposure.

It is generally accepted that pregnancy disruption by reproductive toxicants are mediated by different mechanisms (20, 21). This seems to be the case with the current study as well. The main mechanism of *Icon*®-induced disruption of mid pregnancy is due to a marked potentiation of post–implantation loss. On the other hand, *Icon*® exposure during early gestation is known to interrupt pregnancy both by increased pre- and post-implantation losses (7). In the rat, uterine implantation occurs on day 4 of pregnancy (22). Hence, pre-implantation loss cannot account for any of the anti-reproductive effects seen in this study.

Non-specific and specific mechanisms can potentiate post-implantation loss. Impairment of food intake and maternal weight loss (23) are two of the non-specific factors. In this study, *Icon*® induced maternal weight loss is likely to contribute to the observed anti-gestational effects. Several maternal toxicants interrupt pregnancy (20, 21) and this mode of action is also likely to be operative here as marked toxic signs were evident (ataxia, salivation, exophthalmia, piloerection, depressed body weights, food consumption, production of dark yellow coloured urine and diarrohea)). Stress is another non-specific mechanism that can induce post-implantation losses (24). In rats, stress inhibits luteal function during mid pregnancy causing foetal resorption (25). In this study the *Icon*® treated rats showed features of stress such as piloerection, exopthalmia and this may have contributed to foetal resorption. Other investigators have also shown pyrethroid insecticides like cypermethrine (26) and carbamate insecticides like carbofuran (8) to be producing stress, particularly in mid pregnancy period. Further, we have previously shown increased adrenal weights and enlargement of zona fasciculata of adrenal cortex following *Icon*® treatment (7). Thus, it is likely that stress has played an important role in *Icon*®-induced pregnancy impairment which was seen in this study.

Sedatives are known to interrupt pregnancy (27). Such a mode of action is unlikely in this study as none of the variables of the rat–hole board technique were inhibited: a reliable and a sensitive test used widely to evaluate potential sedatives (15).

A specific mechanism that could increase post–implantation losses in this study is by a direct embryo/foetocidal action of *Icon*®. A marked loss of viability of foetuses (as judged by lack of pulsations in vitelline vessels and presence of dark blue uterine implants at laporatomy) with mid dose and a total lack of foetuses was seen with the high dose. Further, *Icon*® has been shown to kill both human spermatozoa and *Artemia nauplii in vitro* (7), which provide additional

support to the above mechanism. Further, maternal toxic agents consumed during pregnancy are claimed to be embryo/ foetocidal (24) and also several insecticides are known to posses embryo/foetocide activities (8, 10, 12).

In this study, both mid and the high doses of *Icon®* induced moderate to severe vaginal bleeding which was not accompanied with preterm delivery or pups born small for gestational age. However, shortening (28) and prolongation (8, 29) of gestation durations are reported with some pesticides. In contrast, no vaginal bleeding had been reported during early pregnancy of rats following *Icon®* exposure (7) but it did not result in a pre-term delivery or pups born small for gestational age.

Death of foetus in this study may also results from prolonged uterine contractions due to direct myotropic action of *Icon®* on uterine smooth muscle (7) or by an impairment of progesterone release and or progesterone antagonistic activity (30). Indeed, we have previously shown that *Icon®* induced both tonic and phasic contractions in isolated rat uterine preparations (7) and that it has antiprogestrogenic actions (7). An impairment of progesterone release may result due to stress (25) or due to negative effects of hypophagia and weight loss on the generation of GnRH pulses (31).

Foetal deaths could occur with *Icon®* by these mechanisms also. Progesterone/oestradiol ratio plays a crucial role in maintaining viability of embryos (32). Although *Icon®* is not oestrogenic it is anti-progestrogenic (7) and this effect could alter the ratio and make the uterine environment hostile to embryos, leading to their deaths.

This study, together with our previous investigations (7) shows that repeated oral exposure of *Icon®* during both early and mid gestation is detrimental to pregnancy outcome in rats, but induced no detectable gross developmental defects. *Icon®* being an indoor spray, the pregnant domestic animals are liable to get affected. .

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Table 1: Number of rats displaying overt clinical signs of toxicity after oral administration of *Icon*® or vehicle from days 8 -14 of pregnancy

Variable monitored	Control	*Icon*® Treated		
	DW (n=12)	63 mg/kg/body wt. /day (n=12)	83 mg/kg/body wt. /day (n=12)	125 mg/kg/body wt. /day (n=9)
Ataxia	0	1	1	6
Tremor	0	0	0	0
Convulsions	0	0	0	0
Piloerection	0	0	0	2
Exophthalmia	0	0	0	2
Salivation	0	3 (around mouth)	12 (around neck)	12 (whole body)
Lacrymation	0	0	0	0
Coughing	0	0	0	0
Changes in colour of fur	0	0	0	0
Changes in colour of urine	0	0	1 (dark yellow)	6 (dark yellow)
Vaginal bleeding	0	1	3	9
Diarrhoea	0	1	3	9

DW – distilled water

Table 2: Reproductive variables of rats orally administered with *Icon®* or vehicle from days 8 -14 of pregnancy. Data represented as means ± SEM; Ranges in parentheses

Variable monitored	Control	*Icon®* Treated		
	DW (n=12)	63 mg/kg/bodywt. /day (n=12)	83 mg/kg/body wt./day (n=12)	125 mg/kg/body wt. /day (n=9)
Number of corpora lutea	12.2 ± 0.4 (10 - 14)	12.4 ± 0.4 (11 - 14)	11.7 ± 0.6 (8 - 15)	12.5 ± 0.7 (10 - 15)
Number of implants	10.1 ± 0.3 (8 - 12)	9.2 ± 0.4 (7 -12)	7.2 ± 1.0* (0 - 12)	00
Number of viable implants	9.5 ± 0.3 (7 - 12)	8.6 ± 0.6 (5 - 12)	6.0 ± 1.1* (0 - 11)	00
Width of embryo (mm)	9.9 ± 0.6 (8.5 - 12)	10.3 ± 0.2 (9.5 - 11)	9.5 ± 0.0 (8 - 10)	-
Cranio-sacral diameter of embryo (mm)	10.2 ± 0.8 (9 - 13)	10.2 ± 0.7 (8 - 12.5)	10.1 ± 0.8 (8 - 11.5)	-
Gestation length (days)	22.5 ± 0.2 (22- 23)	22.3 ± 0.2 (22 - 23)	22.5 ± 0.2 (22 - 23)	-
Number of pups born	9.3 ± 0.4 (6 - 12)	8.3 ± 0.7 (4 - 12)	5.9 ± 1.1* (0 - 11)	00
Number of live pups	9.1 ± 0.4 (6 - 12)	7.9 ± 0.7 (4 - 12)	5.5 ± 1.0* (0 - 11)	00
Post implantation loss (%)	6.2 ± 3.1 (0 - 36.4)	7.0 ± 3.7 (0 - 44.4)	35.2 ± 11.4 * (0 - 100)	100**
Litter index (%)	92.2 ± 3.7 (54.5 - 100)	88.7 ± 4.5 (55.6 - 100)	64.2 ± 11.2* (0 - 100)	-
Gestation index (%)	930	830	553	-
Live birth index (%)	97.5 ±1.8 (77.8 - 100)	95.5 ± 2.1 (77.8 - 100)	73.0 ± 11.8 (0 - 100)	-
Foetal survival Ratio (%)	90.4 ± 3.5 (54.5 - 100)	84.5 ± 4.6 (55.6 - 100)	59.7 ± 10.6* (0 - 100)	-

As compared with control, *$p < 0.05$, **$p < 0.01$, Mann-Whitney U-test, G-test , - means this information can not be calculated as there were no embryos. DW –distilled water treated.

Table 3: Developmental variables of pups, delivered by rats orally administered with *Icon*® or vehicle from days 8 -14 of pregnancy Data presented as means ± SEM; Ranges in parentheses

Variable monitored	Control DW (n=12)	*Icon*® Treated		
		63 mg/kg/bodywt. /day (n=12)	83 mg/kg/body wt./day (n=12)	125 mg/kg/body wt. /day (n=9)
Body weight (g)	5.0 ± 0.1 (4.3 - 6.0)	5.2 ± 0.2 (4.0 - 6.2)	5.0 ± 0.1 (4.3 - 5.6)	–
Cranial length (mm)	13.5 ± 0.2 (12.5 - 14.5)	13.7 ± 0.2 (12.3 - 14.6)	13.5 ± 0.3 (12.4 - 14.6)	–
Cranial diameter (mm)	10.4 ± 0.2 (9.3 - 12.5)	9.8 ± 0.2 (8.6- 11.0)	9.9 ± 0.2 (9.3- 10.6)	–
Tail length (mm)	15.9 ± 0.2 (14.6 - 17.1)	15.9 ± 0.3 (14.6 - 18)	15.8 ± 0.3 (14.7 - 17.3)	–
Cranio - sacral length (mm)	40.9 ± 0.4 (38.2 - 43)	40.0 ± 0.4 (37.1 - 42.2)	39.7 ± 0.4 (37.9 - 41.6)	–
Fur appearance time (Days)	2.2 ± 0.2 (2 - 3)	2.2 ± 0.2 (2 - 3)	2.3 ± 0.2 (2 - 3)	–
Eye opening time (Days)	15.2 ± 0.3 (14 - 16)	14.8 ± 0.2 (14 - 15)	15.0 ± 0.3 (14 - 16)	–

– means this information can not be calculated as there were no pups. DW – distilled water treated.