

α 71764
✓

WHO/CDS/WHOPES/2001.2
English only
Distr.: General

**REPORT OF THE FOURTH WHOPES WORKING
GROUP MEETING**

**WHO/HQ, GENEVA
4-5 DECEMBER 2000**

REVIEW OF :

**IR3535; KBR3023; (RS)-METHOPRENE 20% EC,
PYRIPROXYFEN 0.5% GR; AND
LAMBDA-CYHALOTHRIN 2.5% CS**



**World Health Organization
Geneva**

© World Health Organization, 2001

This document is not a formal publication of the World Health Organization (WHO) and all rights are reserved by the Organization. The document may, however, be freely reviewed, abstracted, reproduced and translated, in part or in whole, but not for sale nor for use in conjunction with commercial purposes. The views expressed in the document by named authors are solely the responsibility of those authors.

Table of Contents

	Page
1. Introduction	5
2. Review of insect repellent IR3535	9
2.1 Safety assessment	9
2.2 Efficacy - background/supporting documents	11
2.3 WHOPES supervised trials	15
2.4 Conclusions and recommendations	20
3. Review of insect repellent KBR 3023	21
3.1 Safety assessment	21
3.2 Efficacy - background/supporting documents	23
3.3 WHOPES supervised trials	25
3.4 Conclusions and recommendations	28
4. Review of insect growth regulator (RS)-methoprene EC	29
4.1 Safety assessment	29
4.1.1 Effects of methoprene on aquatic nontarget organisms	30
4.2 Efficacy - background/supporting documents	36
4.2.1 Laboratory trials	36
4.2.2 Field trials	40

4.3	WHOPES supervised trials	44
4.4	Conclusions and recommendations	49
5.	Review of insect growth regulator pyriproxyfen GR	50
5.1	Safety assessment	50
5.1.1	Effects of pyriproxyfen on aquatic nontarget organisms	51
5.2	Efficacy - background/supporting documents	53
5.2.1	Laboratory trials	53
5.2.2	Field trials	56
5.3	WHOPES supervised trials	59
5.4	Conclusions and recommendations	66
6.	Review of lambda-cyhalothrin CS for treatment of mosquito nets	68
6.1	Safety assessment	68
6.2	Efficacy - background/supporting documents	69
6.3	WHOPES supervised trials	75
6.4	Conclusions and recommendations	80
7.	General recommendations	82
Annex 1.	References cited	83
Annex II.	List of participants	102

1. INTRODUCTION

The 4th WHOPES Working Group Meeting, the scientific committee to assist the WHO Pesticide Evaluation Scheme (WHOPES) in the review of the reports of testing/evaluation of pesticides in the Scheme, was held in WHO/HQ, Geneva, 4 to 5 December 2000.

The meeting was opened by Dr Lorenzo Savioli, Coordinator, Strategy Development and Monitoring for Parasitic Diseases and Vector Control (PVC). Dr Savioli briefly introduced the structure of the Programme on Communicable Disease Prevention, Eradication and Control (CPE) and that of PVC at the WHO headquarters. He highlighted that prevention through vector control is an integral part of vector-borne disease management and noted the renewed interest in vector control at global level. He informed the participants of the PVC's plans to hold a meeting with WHO Regional Offices in the near future, to revisit the strategic framework for the vector control related activities of the CPE.

Dr Morteza Zaim, Scientist in charge of WHOPES recalled that the first, second and third meetings of WHOPES Working Group have been held in 1997, 98 and 1999, and their reports have been issued as WHO documents and widely distributed. He informed that the present meeting was convened to review the reports of the testing and evaluation of two insect repellents, KBR 3023 (Bayer AG, Germany), IR3535 (Merck, Germany), two insect growth regulators (IGRs) for mosquito larval control, (RS)-methoprene 20% emulsifiable concentrate (EC) (Babolna Bioenvironmental Centre, Hungary), pyriproxyfen 0.5% granule (GR) (Sumitomo Chemical, Japan), as well as lambda-cyhalothrin 2.5% capsule suspension (CS) (Zeneca, UK).

Dr Zaim provided an overview of the Scheme and informed the participants of the role of WHOPES in collection, consolidation and dissemination of information on the use of pesticides for public health. He noted that the recommendations of WHOPES are expected to expedite the registration of pesticides by the Member States. National authorities are encouraged to minimize requirements for local testing of products that have given satisfactory results of trials for similar circumstances. However, Dr Zaim noted that the **WHOPES recommendations are based on the review of the data and information of the products of the above-mentioned manufacturers and do not necessarily apply to nominally similar products of other manufacturer(s), nor to those where the active ingredient is produced by other methods of synthesis.**

Dr Zaim also noted the close collaboration of the Scheme with the International Programme on Chemical Safety (IPCS). Normally WHOPES field studies are carried out only when the human and environment safety of the product has been assessed by IPCS.

Dr Zaim also informed the participants of the meeting of the recent initiative of the Scheme, relating to alternative mechanisms to accelerate the discovery and development of public health pesticides. He briefed the participants on the meeting held in WHO/HQ, Geneva, 18 October 2000, with major manufacturers of public health pesticides and representatives of Malaria Consortium, USNIAID, the Wellcome Trust, the World Bank, in which the great significance of vector-borne diseases and constraints on their control by current methodologies were discussed. The meeting reviewed the future requirements for public health pesticides and unanimously recognized the urgent need to pursue development of alternative pesticide products for vector control, especially as it relates to malaria (treatment of mosquito nets and indoor residual spraying) and dengue/dengue haemorrhagic fever (larviciding and adulticiding). The meeting emphasized

the unique role of WHOPES in coordinating activities related to development of alternative pesticide products for public health and recommended that WHO should visit and discuss with manufacturers of vector control products, under appropriate confidentiality agreements, potential new technologies and compounds for vector control. The meeting requested WHOPES to produce an inventory of potential compounds and technologies available for development and a range of actions for collaborative activities. The report of the meeting is available on WHO homepage on the Internet at >www.who.int/ctd/whopes<.

Once a product is found to meet the requirements of the Scheme, specifications are prepared and published. The specifications include a description of the pesticide concerned and the formulations suitable for use in public health, together with sections concerning their physical and chemical characteristics. If necessary, the maximum contents of impurities are also included in the specifications. Methods for measuring the characteristics of the products are also described. The specifications are part of the International Code of Conduct on the Distribution and Use of Pesticides and are used in international trade and for quality control.

Dr Zaim informed the Group that a memorandum of understanding has been prepared with the Food and Agriculture Organization of the United Nations (FAO) to establish a Joint Meeting on Pesticide Specifications, by which joint FAO-WHO specifications will be developed for technical materials and technical concentrates. Following this new initiative, the WHO specifications for public health pesticides developed do not necessarily apply to nominally similar products of other manufacturers and WHOPES may extend the scope of the specifications to notionally similar products, if it has been satisfied that the additional products are equivalent to those which formed the basis of the evaluation and reference specification.

The meeting was attended by 8 scientists (see list of participants, Annex 2). Professor Arshad Ali, was appointed as Chairman, and Dr Carlo Costantini, as Rapporteur. The meeting was convened in plenary sessions for comprehensive discussion on aspects relating to the public health use of the above-mentioned products and divided into three small working groups to consider the results of the testing and evaluation of different products in detail. The reports of the safety assessments of the International Programme on Chemical Safety (IPCS), WHOPES supervised trials and relevant published literature, as well as the reports submitted by the national disease and vector control programmes (see bibliography, Annex 1) were fully discussed and recommendations on the use of the above-mentioned products were made.

2. REVIEW OF INSECT REPELLENT IR3535

2.1 Safety assessment¹

IR3535 (3-(N-acetyl-N-butyl)aminopropionic acid ethyl ester) is of low acute toxicity, LD₅₀ being >14,000 mg/kg orally and >10 mg/kg dermally. In a 4-week oral toxicity study, no compound-related effects were observed at the highest dose tested, 2700 mg/kg.

In rabbits the “no observed adverse effect level” (NOAEL) in 2- and 4 week studies was 500 mg/kg; at 6000 mg/kg, a decrease in food consumption, and in body weight development were observed at 600 mg/kg in two weeks. In a 90-day dermal toxicity study in rats, the NOAEL was 3000 mg/kg, the highest dose tested. No mortality was observed in rats in a 4-h inhalation exposure to an air concentration of 5.1 g/m³.

In short term studies in rats, mice and dogs, and in a 90-day study in rats, IR3535 showed a low skin irritation capacity. In several studies, 10, 15, and 20% dilutions of, as well as undiluted IR3535 caused marked conjunctival irritation in rabbits; corneal opacities, which recovered slowly, were also observed.

In single studies, IR3535 did not phototoxic or photoallergic reactions in guinea pigs. In limited studies, IR3535 did not demonstrate skin sensitisation potential

IR3535 did not induce developmental toxicity in rats or rabbits in valid studies. In one study in Himalayan rabbits, marked embryotoxicity was observed at dose levels significantly toxic to the does.

¹This assessment is based on the condensed confidential summary of toxicity studies, provided by Merck, Germany, and was performed by IPCS Secretariat.

In a valid 2-generation study in rats, there were no effect of treatment on sperm parameters, oestrous cycles, mating, fertility, duration of pregnancy, numbers of litters or implantations, or growth or development of the pups. In the first generation litters, there was a higher incidence of stillborn pups and pup deaths in the mid and high dose groups; no such finding was observed in the second generation. Because of this inconsistency, the authors considered that this effect was not related to the treatment.

IR3535 was not mutagenic to *S. typhimurium* or *E. coli*, did not induce point mutations (HGPRT) in CHO or V79 cells, or chromosomal aberrations in CHO cells *in vitro* at levels not toxic to the cells. It did not induce micronuclei in bone marrow cells in exposed mice treated intraperitoneally with IR3535 at a dose level 73% of the LD₅₀.

IR3535 is readily absorbed through intact skin in rats: within 24 hours 40% were reported to be absorbed. cultured hepatocytes from rat and man metabolised IR3535 effectively to a single metabolite, the carboxylic acid derivative. Similar metabolite pattern was observed in studies *in vivo* in rats and rabbits. Radioactivity from IR3535 disappears rapidly from the plasma, with a half time of 0.5 - 0.7 h in rabbits and rats after an intravenous administration. The excretion takes place mainly via urine.

After single or repeated skin application, no reactions were observed in human volunteers. Preparations containing IR3535 have been on the market for many years in several countries; no adverse effects have been reported. The producer reports that, upon request, 11 companies using this active ingredient in their products have specifically stated that no adverse effects have been observed.

No long-term toxicity and carcinogenicity studies have been reported. However, consistently negative findings in

genotoxicity testing, together with the apparently innocuous chemical structure of the chemical, make it unlikely that IR3535 were carcinogenic to humans. However, long term toxicity and carcinogenicity studies would be advisable.

2.2 Efficacy - background/supporting documents

Manufacturer internal research (Marchio, 1996) tested IR3535 against a range of nuisance and vector insects in different experimental conditions. Two ml of a 25% (w/v) ethanol formulation of IR3535 was applied on one leg of 6 subjects, giving an approximate 0.6 mg/cm^2 target dose, while the other leg was kept as a control. Tests were carried out outdoors for 6 nights with different subjects and up to 6 hours after treatment in two riverine villages in Liberia between 1900 and 0700 h. The percentage reduction of malaria vectors *Anopheles gambiae* and *An. funestus*, which constituted ~96% of the 1,157 mosquitoes collected landing on the control human baits, was 92% after 6 hours.

The time until a second bite from 500 *Aedes aegypti* freed in a 43-dm^3 cage was measured when three increasing target doses (0.1, 0.2, and 0.3 mg/cm^2) were applied on the forearm of subjects who exposed them for 5 minutes every hour. The length of action was directly correlated with dose, plateauing off at about 7–8 hours from application with the two higher doses tested. A similar experiment comparing a 30% formulation of IR3535 to a 33% formulation of DEET on 10 test subjects produced mean protection times of 7.6 and 6.3 hours, respectively.

Immediate and long term effectiveness against *Pediculus humanus* was evaluated under laboratory conditions by measuring the escape response of 96 adult lice put in a 1x1 square tissue treated with 20% IR3535. A total of 94% left the treated tissue after 32 minutes, 71% reaching the outer ring of the arena 32 cm from the treated centre, whereas more than 90% of the control batch remained on the central tissue. Soaking the

square tissue with IR3535 48 h before the test, still produced an escape response in 90% of the exposed lice. In Madagascar, three groups of schoolchildren aged 6 to 16 years with similar lice infestations were treated with an insecticidal shampoo and then applied 20% ethanolic formulations of IR3535 or DEET, or otherwise left untreated as a control. On the seventh day after treatment the control group had an infestation about 50% of the pre-treatment value, whereas in the other two groups this was less than 2%.

Czech Republic – An ethanol formulation of IR3535 was compared to DEET both in the laboratory and in the field (Rettich, 1999). Cohorts of 50 nulliparous *Ae. aegypti*, 7–14 days old, kept in a 12-dm³ cage were exposed for 1–3 minutes to arms of 4 male and 4 female subjects who treated their left forearm with IR3535 and the right forearm with DEET. Repellents were applied at increasing doses in different experiments: 0.15 mg/cm² (34 tests), 0.30 mg/cm² (7 tests), and 0.45 mg/cm² (14 tests). The number of biting mosquitoes was recorded immediately after application and at 0.5, 1, 1.5, 2, 3, and 4 hours intervals. When the biting activity on an untreated arm exposed before and after each test was lower than 0.5 bites/second, the test was not executed or was discarded.

As expected, the average number of bites increased with time from application at all doses tested and for both repellents. The arm treated with IR3535 received on average 1.6x–20.8x more bites than that treated with DEET. This difference decreased with time and reduced to zero at 4 hours from application at the highest dose tested.

Field tests were carried out in a flood-plain forest in Central Bohemia where biting rates of about 714 landings/person/hour by *Ae. cantans* and *Ae. annulipes* were measured during landing collections on untreated human baits for 1–5 minutes just before repellent application. Five ml of a 15% ethanol solution of IR3535 or DEET were applied on the legs (from hips to ankles) of subjects, giving an application target dose of ~0.125 mg/cm². The human baits collected biting mosquitoes off their legs for 5

minutes, then moved 5–10 m away and repeated so in 2–3 sites in the forest. The tests lasted until the repellent effect faded away or up to 6 hours from application. During resting periods, the subjects slowly walked outside the forest where biting was negligible. Maximum likelihood re-analysis of data showed significant differences between the two repellents, giving approximate 95% protection times of 4.8 and 9.7 hours for IR3535 and DEET, respectively.

Thailand – An ethanol formulation of IR3535 was compared to DEET both in the laboratory and in the field (Thavara *et al.*, unpublished report). In laboratory tests, 0.1 ml of a 20% (w/w) solution of each repellent was applied at the start of a test onto a 30-cm² marked area on either forearm of three subjects 23–35 years old. This treatment achieved a target dose of 0.55 mg/cm². The treated skin was exposed for 3 minutes every half an hour to 250 females of disease vectors *Ae. aegypti*, *Culex quinquefasciatus*, *Cx tritaeniorhynchus*, or *An. dirus*, kept in a 27-dm³ cage. Depending on the biting cycle of each species, tests were performed for eight hours at corresponding times during the day or night. Protection time was defined as the time elapsed from the application of repellent and the second bite. IR3535 had the same median protection time as DEET for culicine species, but about half (46%) the median protection time of DEET for *An. dirus*, a significantly lower score compared to either the culicines or DEET.

Field tests at five selected peninsular and continental sites were carried out over 5 months with six pairs of human baits 18–42 years old collecting mosquitoes biting or landing on their legs: 3 ml of the same formulation used in the laboratory tests was applied on one leg of six collectors, whereas the remaining six collectors acted as controls. The other leg of the treated collectors was applied with 3 ml of an identical DEET formulation. This application protocol produced a target dose of approximately 0.3 mg/cm². Collections alternated 10-min catching bouts with 10-min resting bouts carried over for up to

eight hours at day (0900–1700 h) or five hours at night (1900–2400 h). The percentage reduction in mosquitoes landing on the treated collectors as compared to the control collectors was calculated at hourly intervals.

Aedes albopictus accounted for 76% of 1,083 mosquitoes caught in two days by the untreated collectors during the daytime tests, giving an average biting rate for this species of 17.1 landings/person/hour. Other predominant species caught by the control collectors were *Armigeres subalbatus* (13%) and *Coquillettidia crassipes* (11%), giving biting rates of 3.1 and 2.0 landing/person/hour, respectively. Only 23 *Ar. subalbatus* could be caught by the treated collectors during these tests, giving a specific percentage reduction of 94% and 90% for IR3535 and DEET, respectively.

During the night-time tests, 1,076 mosquitoes belonging to 12 species were caught in 6 days by the control collectors. Of these, *Cx gelidus* accounted for 32% of the total, *An. hyrcanus* for 16%, *Cx quinquefasciatus* for 14%, and *An. minimus* for 13%. The remaining 25% was composed by *Cx tritaeniorhynchus*, *Mansonia dives*, *Cx sitiens*, *Ma. annulifera*, *Ma. annulata*, *An. maculatus*, *An. sawadwongporni*, and *An. pseudowillmori* in decreasing order of frequency. Another 49 mosquitoes of unspecified species were also collected. The species-specific and overall biting rates varied widely from one site to another, ranging 0.5–23.2 and 3.7–36.3 landings/person/hour, respectively. IR3535 and DEET-treated collectors caught 6 and 7 mosquitoes during the tests, belonging to *An. hyrcanus*, *An. minimus*, and *Cx sitiens*, giving overall percentage reductions of 99.5% and 99.4%, respectively. There was no significant difference at each site in the percentage reduction of IR3535 compared to DEET. When comparing different sites, however, it appeared that IR3535 efficiency was significantly reduced where anophelines were the most abundant species.

In both field experiments there was no clear decrease in the percentage reduction with time, presumably due to the small

sample sizes obtained. No rash, skin irritation, or hot sensation was reported by the subjects treated with IR3535 during and after its application. The main conclusions of this study are: i) under conditions of low biting pressures IR3535 performs equally well than DEET for up to 5 hours in the case of several culicine species belonging to the genera, *Culex* and *Mansonia*, and up to 8 hours against *Ae. albopictus*; ii) as in the case of DEET, IR3535 performs comparatively worse against *Ar. subalbatus* and several other South-East Asian anopheline species.

2.3 WHOPES supervised trials

Burkina Faso – The protection time of IR3535 against bites from *An. gambiae* complex mosquitoes was compared to that of DEET in field studies carried out for six months throughout one rainy season in a rural village near Ouagadougou (Costantini & Ilboudo-Sanogo, unpublished report). Eight human subjects applied four target doses (0.10, 0.30, 0.60, and 0.80 mg/cm²) of an ethanolic formulation of either repellent on their lower limbs just before the start of the trials. Four groups of two collectors each were allocated to a 4x4 (sites x nights) latin square which was replicated six times for each of the target doses tested. Each group tested one repellent on any one night, whereas one group of collectors acted as a placebo (ethanol only) control; the remaining fourth group tested another repellent (see below). All mosquitoes landing on the exposed legs and feet were aspirated out of doors from 1800 to 2200 h, and indoors from 2400 to 0400 h, with a resting pause of two hours in-between, thereby allowing evaluation of protection efficacy for a period of up to 10 hours.

Almost 30,000 mosquitoes belonging to 15 species (or species complexes) were caught by the control collectors during 96 test nights, giving an average landing rate of 19.2 landings/person/hour. About 93% were *An. gambiae* s.l., followed by *An. nili* (3.8%), *An. funestus* (1.3%), *An. pharoensis*

(0.2%), and several *Aedes* species, among which important vectors of dengue and yellow fever. As expected, median protection times for *An. gambiae* s.l. increased with the application dose, ranging 4.6–22.6 hours for IR3535, and 5.2–14.7 hours for DEET. At the 90% endpoint, protection times ranged –2.0–6.7 hours for IR3535 (the negative value indicating a reduction <90% immediately after application, as extrapolated from the maximum likelihood linear model relating the percentage reduction of mosquitoes landing on the treated subjects compared to the control subjects) and 2.1–10.9 hours for DEET. Thus, protection times were generally longer for DEET than IR3535.

The linear model relating the log-dose at application with the 90% protection time allowed estimation of the repellents' loss rate, and their ED₉₀ (effective dosage). Loss rates of the two repellents were similar, whereas the ED₉₀ was more than twice higher for IR3535 than DEET. This was confirmed by laboratory trials on the F1 progeny of wild females using the "separate arms" protocol of Curtis *et al.* (1987), which gave a 0.032 relative potency estimate for IR353 compared to DEET. The proportion of *An. gambiae* s.l. harbouring sporozoites in their salivary glands (estimated by ELISA) was not significantly different across treatments, indicating that a reduction in the number of bites afforded by the repellents reflected a reduction in the number of infective bites as well. Perception of the repellents cosmetic properties by the test subjects revealed a better assessment for IR3535, which was never deemed irritant and its odour was acceptable instead of unpleasant most of the time.

Malaysia – An ethanol formulation of IR3535 was compared to DEET both in the laboratory and in the field (Yap 1998 a,b). In laboratory tests, 0.18 ml of a 25% (w/v) solution of each repellent was applied at the start of a test onto a 90-cm² area on the right forearm of human subjects. This treatment achieved a target dose of 0.50 mg/cm². The left arm was consistently left untreated as a control. Both arms were contemporarily offered

for 3 minutes to 50 *Ae. albopictus*, or 200 *An. dirus*, aged 3–10 days kept in a 216-dm³ cage, by exposing an area of 25 cm² of each arm. The number of mosquitoes landing or biting was counted 1, 2, 4, 6, and 8 hours after the application of the test samples. A fresh batch of starved females was introduced at every assessment hour. The same procedure was followed for the three tests comparing an untreated arm vs. either a placebo (an arm treated with 75% ethanol), an IR3535-treated arm, or a DEET-treated arm. Three replicates (i.e. test days) were performed for each species. The percentage reduction in mosquitoes landing on the treated arm as compared to the control arm was calculated for every testing bout.

A maximum likelihood re-analysis of the data showed that IR3535 had a median protection time of 5.8 hours and a 95% protection time of 1.9 hours against *Ae. albopictus*. Both estimates were 10% and 26% lower than those of DEET. The median protection time was 5.3 hours for *An. maculatus*, but the 95% endpoint could not be calculated, because even extrapolation of the functional relationship at time of repellent application gave a percentage reduction less than 95% (74%). The median protection time was 32% lower than that afforded by DEET. Thus, the response of the two species was substantially different, albeit in both cases IR3535 produced a lower percentage reduction than DEET. The difference in efficacy between the two repellents, however, was greater in the case of *An. maculatus*. Moreover, this species had longer median protection times but fairly lower percentage reduction estimates than *Ae. albopictus* for both DEET and IR3535 at the dose tested.

A similar protocol and the same repellent formulations as for the laboratory tests were employed during field tests carried out over three days in two coastal areas of north-western peninsular Malaysia harbouring high frequencies of *Ae. albopictus* (forest area) and *Cx quinquefasciatus* (urban area). A total of 0.75 ml and 1.5 ml of repellent formulation or placebo were applied on the right arm (wrist to elbow) and leg (knee to ankle),

respectively, of human baits instructed to protect all other areas of the body from mosquito bites and to stay at least at 5 m from each other. The application achieved an approximate target dose of 0.20 mg/cm². In the forest area, 9 human baits were involved (3 treatments x 3 repetitions of each treatment), whereas in the urban area 18 subjects were involved (3 treatments x 3 repetitions of each treatment x 2 groups). The efficacy of the repellents was tested over up to 7.75 hours, with collections performed during 45 minutes 0, 1, 3, 5, and 7 hours after application (from 0900 to 1645 h in the forest area, and from 2100 to 0045 h in the urban area). In the urban area, one group of human baits applied the samples 4 hours before the start of the tests to gather data on the sixth to eighth hours post-treatment.

In contrast with expectations, due to unfavourable climate *Ae. albopictus* comprised only about 49% of the 1,963 mosquitoes collected in the forest area, the remaining 51% accounted mainly by *Cx bitaeniorhynchus*, *Cx gelidus*, *Cx sitiens*, *Cx tritaeniorhynchus*, and to a lesser extent by *Ae. gardneri*. From the data presented it is not possible to extrapolate results for each species, therefore—even if this is not the legitimate procedure—results were considered for the pool of species. Biting rates during the experiment averaged 42.4 landings/person/hour. Only 17 and 7 mosquitoes were caught after 5 hours from repellent application by the IR3535- and DEET-treated collectors, giving a cumulative percentage reduction of 96.3% and 98.2%, respectively. In the urban area, *Cx quinquefasciatus* comprised ~91% of the 2,514 mosquitoes caught during the experiment, giving an average biting rate of 52.0 landings/person/hour. Other species caught were *Cx gelidus* (7.5%), and *Ae. aegypti* / *Ae. albopictus* (1.7%). Only 3 and 1 mosquitoes were caught after 5 hours from application by the IR3535- and DEET-treated collectors, giving a cumulative percentage reduction of >99% for both repellents.

USA – Five subjects assessed the protection time of IR3535 compared to DEET in the Everglades National Park, Florida,

against attack from *Ae. taeniorhynchus* (Barnard, unpublished report). On each test, two human baits applied 1 ml of a 25% formulation of either repellent on 650 cm² of skin surface of one arm, chosen at random. This treatment achieved a target dose of 0.38 mg/cm². Another two subjects applied different repellents. The last subject acted as a control, treating the arm with an ethanol placebo. All body parts other than the treated arm were protected with appropriate clothing, boots, gloves, and head-nets. At hourly intervals, treated subjects collected mosquitoes landing off their arms for 3 minutes during six hours. Control subjects only collected for one minute, their yield was subsequently adjusted by 3x multiplication. During five tests performed over three days, when all human subjects received each treatment once, a total of 1,462 *Ae. taeniorhynchus* were collected. Two endpoints were assessed from the data, namely percentage reduction in landing mosquitoes on treated collectors compared to control collectors (i.e. percent repellence), and complete protection time defined as the time elapsed between repellent application and the observation period immediately preceding that in which the first mosquito landing on treated skin was observed.

Mean landing rates on control collectors generally decreased across each test, ranging 184–2,532 landings/person/hour. Both repellents provided ≥89% repellence throughout the test, hence median protection times extrapolated from the data-set are highly imprecise. Maximum likelihood re-analysis provided median protection time estimates higher for IR3535 than DEET (9.3 vs. 7.1 hours, respectively), but at the 95% end point the reverse was true (4.9 vs. 5.4 hours). Mean complete protection time was significantly higher for DEET (5.2 hours) than IR3535 (3.0 hours) using parametric ANOVA, but re-analysis of the data-set using survival analysis procedures showed that median complete protection time of IR3535 (3 hours) was just marginally lower than that of DEET (6 hours) employing Cox's F-test ($P=0.05011$).

2.4 Conclusions and recommendations

1. Although IR3535 has been in the market for more than 20 years, there are no reports of adverse effects on human health. Based on current information, chemical structure and mode of action, it is unlikely that IR3535 is hazardous to humans or, when used as an insect repellent, to the environment. Because of the eye-irritating potential, appropriate labelling of IR3535-based products to prevent eye exposure is recommended.
2. Ethanolic preparations of IR3535 showed good repellent properties under temperate and tropical conditions. The manufacturer's recommended target dose of 0.3 mg a.i./cm² of skin achieves 95% protection for approximately 2-3 hours against several *Aedes* and *Culex* mosquito species. Additional studies are recommended to further assess the repellent properties of IR3535 against anophelines as some of the studies under review showed its lower efficacy as compared to culicines.
3. IR3535 is recommended as a safe and effective insect repellent for human use.

3. REVIEW OF INSECT REPELLENT KBR 3023

3.1 Safety assessment²

KBR 3023 (1-piperidinecarboxylic acid 2-(2-hydroxyethyl)-1-methylpropylester) is of low acute toxicity in rats (LD₅₀: 4743 mg/kg body weight) and mice after oral administration and in rats after dermal (LD₅₀: >5000 mg/kg body weight) and inhalation exposure (LD₅₀: >4364 mg/kg body weight).

The chemical was demonstrated to have negligible dermal and limited ocular irritation capacity in rabbits. In the different skin sensitization tests, KBR 3023 remained negative. Phototoxicity was studied in 50 persons, and no phototoxicity was observed.

Upon repeated administration, the changes observed at the lowest doses, were induction of the hepatic cytochrome P450-dependent reactions, concomitant with an increased relative liver weight. In a 13-week study, low toxicity was observed. Locally skin changes that subsided after the cessation of the treatment, were observed in all treated groups, i.e.- even at the lowest dose of 80 mg/kg/day.

No carcinogenic activity was observed in a long-term dermal study in rats, in which at the highest dose, after two years, cystic degeneration of the liver was observed. In this study dermal effects similar to those seen in the 13-week study, but no other (except those in the liver) systemic effects, were observed.

No neoplastic response, related to the treatment, was observed in a long term study using mice. However, the study has limited power, since no treatment-related effects on any parameter were observed, i.e., it is not clear that the MTD was reached.

² This assessment is based on the condensed confidential summary of toxicity studies, provided by Bayer AG, Germany, and was performed by IPCS Secretariat.

No reproductive toxicity was observed in a 2-generation study, using dermal application, at a daily dose of 200 mg/kg body weight. In an oral embryotoxicity and teratogenicity assay, no teratogenicity, but slight retardation of skeletal development was observed at a dose that was toxic to the dams (500 mg/kg body weight/day). In a dermal teratogenicity study with rats, no signs of embryotoxicity or teratogenicity was observed at a daily dose of 400 mg/kg, which was the highest dose that could be applied dermally; this dose level induced an increase in the relative and absolute liver weight in the dams, but no signs of overt toxicity. In a range-finding teratogenicity study in rabbits, at a dose of 1000 mg/kg body weight/day, that induced mortality in dams, decreased pregnancy rate was observed. In the main study, a dose level of 200 mg/kg body weight/day was used. No treatment-related effect on the pregnancy outcome was observed; the number of dams with soft feces was increased at a dose level of 200 mg/kg body weight/day.

Genotoxicity was studied using *Salmonella typhimurium* point mutation tests with 4 different strains, with and without metabolic activation, Chinese hamster ovarian cells hgp_rt-assay, and clastogenicity assays (chromosomal aberrations) with CHO cells, and with bone marrow micronucleus cell investigation *in vivo* in mice, as well as using the unscheduled DNA synthesis test on rat primary hepatocytes. No indication of genotoxicity was observed although concentrations that were cytotoxic (or induced mortality in case of the *in vivo* study) were used, with the exception of the chromosomal aberration test which gave a positive response at a cytotoxic concentration.

In acute (2000 mg/kg bw dermally) or subchronic (200 mg/kg bw/d) no sign of behavioural or pathological anatomical neurotoxicity was observed.

After intravenous administration of KBR 3023, ¹⁴C labeled in the hydroxyethyl moiety, some 80-95% of the radiolable was recovered either in the urine (75-90%) or in faeces 5-16%,

mostly as hydroxylated metabolites, within 48 days. Following dermal application, a dose-dependent absorption was observed in rats, with approx. 60% absorbed at low (20 mg/kg) dose level, and 40-55% at a high (200 mg/kg) dose level.

In dermal absorption studies in healthy male volunteer humans, 15 mg of KBR 3023 were applied on the skin either as the undiluted technical grade material or as a 15% ethanol solution; no occlusion was used. Urine and faeces were collected for six days after the 8-h application. In marked contrast to rats, only 2-4% of the applied radioactivity was recovered in urine. No metabolites were observed in human urine that were not found in rats and that the metabolite patterns were similar in rats and in humans.

The study on the acute toxicity of KBR 3023 on *Daphnia magna* and rainbow trout in a static test were performed according the guidelines of OECD, and conducted in compliance with OECD GLP standards in a certified laboratory. Effective concentrations for *Daphnia magna* and lethal concentrations for *Oncorhynchus mykiss* were in excess of 100 mg/L, and thus indicate low toxicity.

3.2 Efficacy - background/supporting documents

Czech Republic – An ethanolic formulation of KBR 3023 was compared to DEET both in the laboratory and in the field (Rettich, 1999). Experimental procedures were described previously in the section concerning IR3535 testing. In 138 laboratory tests, at the lower dose of 0.15 mg/cm² KBR 3023-treated subjects received 53–88% of the average number of bites received by the DEET-treated subjects. Biting activity of *Ae. aegypti* commenced for both repellents after 1 hour of testing. In 42 tests at the higher dose of 0.30 mg/cm², however, KBR-treated subjects received 1.7–2.5 more bites than DEET-treated subjects, and biting activity commenced 0.5 hours in advance for KBR 3023 compared to DEET.

During 3–4 field tests in a flood-plain forest in Central Bohemia experiencing high biting rates of *Ae. cantans*, *Ae. annulipes*, and *Ae. sticticus* (1,200–2,400 landings/person/hour), two human baits were treated with 2 or 5 ml of 20% KBR 3023 or 20% DEET, giving approximate target doses of 0.07 and 0.17 mg/cm². Considering a constant biting rate of 100 landings/person every 5 minutes, maximum likelihood estimates did not evidence significant differences between the two repellents, giving approximate 95% protection times of 2.3 and 5.4 hours for doses of 0.07 and 0.17 mg/cm², respectively.

Malaysia – KBR 3023 protection time against day-biting from *Ae. albopictus* and night-biting from *Cx quinquefasciatus* was compared to DEET outdoors in a forested orchard and indoors in an urban squatter of peninsular Malaysia (Yap *et al.*, 1998). Amounts of 0.5 and 1 ml of 10% and 20% ethanolic formulations of KBR 3023 and DEET were applied on the right arm (wrist to elbow) and leg (knee to ankle) of eight human baits positioned at least 5 m away from each other. Such treatment achieved approximate target doses of 0.07 and 0.15 mg/cm², respectively. The left limbs were left untreated as controls. During the day-time study, collections of landing/biting mosquitoes were performed for 8 hours from 0900 to 1700 hours, whereas in the night-time study collections lasted four hours (2100–0100 h). Thus, in order to assess the repellents efficacy after 8 hours from application, two groups of 8 collectors each were employed in the latter experiment, and the first group applied the repellents four hours before the start of each trial.

Three replicates of each experiment yielded a total of 5,525 and 6,633 mosquitoes giving average landing rates on the control limbs of 28.8 and 34.5 landings/person/hour, respectively. *Aedes albopictus* constituted about 89% of the total catch from the orchard, the other species collected belonging to the genera *Armigeres* (~9%), *Aedes* (~1.2%), *Culex* (~0.6%) and *Mansonia*

(0.01%), whereas *Cx quinquefasciatus* represented more than 99% of the total catch from the urban squatter.

A maximum likelihood re-analysis according to the model proposed by Rutledge *et al.*, (1985), assessing the functional relationship of the decrease with time in the percentage reduction of mosquitoes attempting to land/bite the treated collectors, showed consistently longer protection times of KBR3023 than DEET in both experiments. Both repellents showed longer protection times for *Cx quinquefasciatus* than *Ae. albopictus*. Tested formulations did not cause any problem of skin irritation or other adverse effects during and after application; moreover, the human subjects reported to be much more comfortable with the odour of KBR 3023 than that of DEET.

3.3 WHOPES supervised trials

Burkina Faso – The protection time of KBR 3023 against bites from *An. gambiae* complex mosquitoes was compared to that of DEET in field studies carried out in a rural village near Ouagadougou (Costantini & Ilboudo-Sanogo, unpublished report). Experimental procedures and general results were presented previously in the section concerning IR3535 testing.

As expected, median protection times for *An. gambiae* s.l. increased with the application dose, ranging 4.6–183.7 hours for KBR 3023, and 5.2–14.7 hours for DEET. At the 90% endpoint, protection times ranged 3.2–38.7 hours for and 2.1–10.9 hours for DEET. The protection times of KBR 3023 at the two higher doses tested were highly imprecise, as they were extrapolated from the maximum likelihood linear model relating the percentage reduction of mosquitoes landing on the treated subjects compared to the control subjects, and only a few mosquitoes were caught by the KBR 3023 treated collectors. In

any case, protection times were always longer for KBR 3023 than DEET.

The linear model relating the log-dose at application with the 90% protection time allowed estimation of the repellents' loss rate λ , and their ED₉₀. The loss rate of KBR 3023 was lower than DEET, whereas the ED₉₀ of DEET was lower than that of KBR 3023. This ranking was confirmed by laboratory trials on the F1 progeny of wild females using the "separate arms" protocol of Curtis *et al.* (1987), giving a 0.792 relative potency of KBR 3023 compared to DEET. The proportion of *An. gambiae* s.l. harbouring sporozoites in their salivary glands (estimated by ELISA) was not significantly different across treatments, indicating that a reduction in the number of bites afforded by the repellents reflected a reduction in the number of infective bites as well. Most of the time the human subjects deemed the odour of KBR 3023 acceptable instead of unpleasant as in the case of DEET.

Scotland – Experiments were carried out in summer 1999 in a peat marsh in Argyllshire where high densities of the Scottish biting midge *Culicoides impunctatus* can substantially impair outdoor activities (Mordue, unpublished report). Five subjects applied on one arm two target doses (0.17 and 0.30 mg/cm²) of a 20% w/v KBR 3023 formulation, leaving the other arm untreated as a control. Experiments were performed during 31 test nights between 1930 and 2230 h, 0, 2, 4, 6, and 8 hours after repellent application. After allowing 10 minutes for build up of the midge population, landing parous female *C. impunctatus* were pooted off the arms of a subject acting as bait by two collectors for 3 minutes. Repeated exposures of the five human baits (4 replicates) allowed comparison of between-subjects variation in response to the repellent, and the decrease of effectiveness with time. A larger sample of ten subjects was used on one replicate to establish a more general response at time zero from application.

Immediately after application, 0.30 mg/cm² of KBR 3023 afforded a ~79% significant reduction in landing midges as compared to the control arm. There was no significant difference in response between the five subjects tested. The repellent effect at this application dose declined to zero after about 7 hours. The decline with time was substantially slower at the 0.30 mg/cm² dose. Biting rates could be established only once on the basis of the inflammatory reaction on the skin of both arms: the lower proportion of bites (5/142) compared to landings suggested that not all the midges that landed on the treated skin would have completed the behavioural sequence leading to the taking of a blood meal. Moreover, the extremely high landing rates on control arms (up to 40,000 landings/person/hour) reduced the pootering efficiency. The efficacy of KBR 3023 in preventing bites, therefore, may have been underestimated in this experiment.

USA – The protection time of KBR 3023 against attack from *Ae. taeniorhynchus* was compared to that of DEET in the Everglades National Park, Florida (Barnard, unpublished report). Experimental procedures and general results were presented previously in the section concerning IR3535 testing. Both repellents provided ≥89% repellence throughout the test, hence median protection times extrapolated from the data-set are highly imprecise. Maximum likelihood re-analysis provided median protection time estimates higher for KBR 3023 than DEET (8.5 vs. 7.1 hours, respectively); this ranking was maintained at the 95% end point (6.1 vs. 5.4 hours). Parameter estimates of the functional relationship relating percent repellence with time from application, however, were not significantly different. Median complete protection times were identical for DEET and KBR 3023 (6 hours).

3.4 Conclusions and recommendations

1. KBR3023 has a good safety profile and cosmetic properties. Alone or in typical formulations it does not significantly attack common household materials including plastics, coatings, foils, and varnishes.
2. KBR3023 was tested under temperate and tropical conditions against important disease vectors *Ae. albopictus*, *An. gambiae* and *Cx quinquefasciatus* and several pest mosquito demonstrating excellent repellent properties comparable to, and often superior, to those of the standard DEET.
3. At the manufacturer's recommended target dose of 0.3 mg a.i./cm² of skin, KBR3023 confer more than 95% protection up to 6-7 hours after application. At comparable doses, KBR3023 showed significantly longer protection times than DEET against *An. gambiae* complex malaria vectors; although further studies are needed to assess its efficacy against a broader range of anopheline vector species, KBR3023 can be recommended as the repellent of choice for malaria prevention.
4. Given the promising results shown by KBR3023, efficacy test of this chemical for treatment of mosquito nets, garments, and other materials is recommended.
5. KBR3023 is recommended as a safe and effective insect repellent for human use.