

CRP D62008, Meeting Code: RC-1143.1

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WORKING MATERIAL

The Development of Generic Irradiation Doses for Quarantine Treatments

REPORT OF THE FIRST RESEARCH COORDINATION MEETING

Vienna, 5-9 October 2009

FAO / IAEA Division of Nuclear Techniques in Food and Agriculture

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Vienna, Austria, 2009

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INTERNATIONAL ATOMIC ENERGY AGENCY

**Report of the First Research
Coordination Meeting**

On

**The Development of Generic Irradiation Doses
for Quarantine Treatments**

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1. Introduction

The first Research Coordination Meeting (RCM) for the Research Coordination Project (CRP) on the Development of Generic Irradiation Doses for Quarantine Treatments met at the IAEA Headquarters in Vienna, Austria, from 5-9 October 2009.

The Meeting recalled that the project will establish validated irradiation doses for non-fruit fly species of quarantine significance. The project results will strengthen existing irradiation standards developed under the International Plant Protection Convention (IPPC), thereby allowing international trade for various fruits and vegetables through the use of generic irradiation doses for a wide range of quarantined pests.

The Meeting was chaired by G Hallman, and the scientific secretaries were C. Blackburn and A. Parker. The list of participants (Annex A) and adopted Agenda (Annex B) are attached.

2. Background

Regulatory authorities and scientists from many internationally recognised institutions have generated research data on the effectiveness of irradiation as a quarantine treatment against a range of insect pests infesting various fruits and vegetables. These authorities have concluded that the development of generic irradiation doses are both feasible and desirable as they could, in many cases, negate the need to develop or validate specific irradiation doses tailored to individual arthropod species.

The use of gamma ray, electron beam and X-ray irradiation as a plant health (phytosanitary) treatment has expanded rapidly in recent years. This application of irradiation technology is important for both developed and developing countries due to uncertainties on the future availability and increasing price of methyl bromide, a fumigant facing increasing restrictions under the Montreal Protocol, but still widely used as a quarantine and pre-shipment treatment for pests of quarantine significance. There is a need for validated alternative post-harvest pest control methods, such as ionising radiation, and the time is right for applied research leading to the development of additional irradiation treatments.

Since 1981, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has been supporting research, education and cooperation in the use of ionising radiation as a post-harvest phytosanitary treatment. In this regard, the Food and Environmental Protection Sub-programme has implemented four coordinated research projects in the area of phytosanitary applications of irradiation. These projects established the basis for developing national and international standards on the use of irradiation as a phytosanitary (quarantine) treatment. Specifically, in 2003 the IPPC approved the International Standard for Phytosanitary Measures (ISPM) Guidelines for the Use of Irradiation as a Phytosanitary Measure (ISPM 18), which facilitated international trade in irradiated fresh fruit. In 2009, the IPPC also adopted eight irradiation treatments for various insect pests, including a generic treatment for fruit flies of the family Tephritidae, for inclusion in ISPM 28 on Phytosanitary Treatments for Regulated Pests.

Despite these successes, important gaps in knowledge still remain, and there are a number of other critical non-fruit fly pests of quarantine significance where comparatively little research on their susceptibility to irradiation has been performed. Such pests include mites, thrips, mealybugs, weevils, leaf miners, aphids and scale insects.

A Consultants Meeting held at the IAEA Headquarters from 3 - 7 November 2008 considered these gaps in knowledge. The purpose of the Consultants Meeting was to advise the Food and Environmental Protection and the Insect Pest Control Subprogrammes of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture (NAFA) on the proposed Coordinated Research Project (CRP) on the Development of Generic Irradiation Doses for Quarantine Treatments. The Meeting recommended that:

- i. A set of guidelines should be developed during the first RCM on the application and reporting of dosimetry to ensure consistency.

- ii. Research protocols should be developed during the first RCM that include, among other things, definitions of the measure of efficacy for irradiation as a phytosanitary option, for all the arthropod groups that will be studied under the CRP.
- iii. The CRP outcomes should facilitate the finalisation of IPPC treatments and standards that deal with phytosanitary applications of irradiation.
- iv. A high priority should be given to develop a generic dose for all phytophagous mites.
- v. A generic dose for all weevils should also be developed.
- vi. Reducing the generic dose of 400 Gy for all Insecta (except pupae and adults of Lepidoptera) should be investigated.
- vii. The CRP framework should consider the outputs of previous CRP and synergies with related TC country and regional irradiation projects.
- viii. Large scale testing up to 30 000 insects should be considered in confirming that the selected dose is efficacious.

The first Research Coordination Meeting noted that three of the recommendations could not be fully addressed, namely:

- Recommendation (iv): Although the research will start to establish a body of work that could eventually result in a generic dose for the quarantine treatment of all mites, the CRP includes work on four species of phytophagous mites, and it is unlikely that a generic dose could be agreed based on four species.
- Recommendation (v) cannot be addressed as none of the CRP participants are studying weevils.
- Recommendation (vi): In making this recommendation, it was assumed that the 400 Gy generic dose for all Insecta (excluding pupae and adults of Lepidoptera) would be accepted by the IPPC. However, this specific treatment remains one of the six irradiation quarantine treatments that are currently undergoing further review and consideration by the IPPC for future adoption.

3. Objectives of the Coordinated Research Project

The overall objective of the CRP is to validate generic treatment doses for groups of arthropod pests of quarantine significance in international trade. Secondary objectives include an examination of the effects of low oxygen commodity storage and dose rate on efficacy and commodity tolerances.

Research will assist in the development of a generic dose treatment for Insecta (except for pupae and adults of Lepidoptera). The work will also assist in setting doses for the quarantine treatment of phylum Arthropoda (and a few subgroups within that phylum) as well as directly establish minimum doses that will provide quarantine security against specific pests in various commodities.

Research on specific non-fruit fly pest species or groups will be conducted at different locations by researchers using practices that are adequate for phytosanitary applications of irradiation, such as accurate, traceable dosimetry, acceptable pest-rearing methods and precise determinations of efficacy. Efficacy under commercial conditions of oxygen stress, whether intentional or passive, will be tested for certain applications. Tolerances of specific commodities under various commercial conditions will also be studied.

Expected Research Outputs

- Data on applications of irradiation on pests of quarantine significance.
- Validation of irradiation doses for the quarantine treatment of specific insect species.
- Determination of the effect of low oxygen gas content (i.e. modified atmosphere storage) and dose rates on irradiation efficacy.
- The tolerance of specific products to irradiation.
- Communication of research findings to the wider scientific community.

Expected Research Outcomes

- Consideration of the project findings by the IPPC and national plant protection organisations.
- Additions to International Standards for Phytosanitary Measures, including ISPM 28 on Phytosanitary Treatments for Regulated Pests.
- Beneficial outcomes to developed and developing countries by increasing international trade in high value crops and fruits that are subjected to irradiation treatments.
- An increase in international trade and a lowering of trade barriers by addressing quarantine requirements for insect pests.

The meeting noted that one of the considerations that differentiate irradiation from other phytosanitary treatments is the fact that quarantine irradiation doses may not cause acute mortality (but prevent further development and/or reproduction) and quarantine inspectors are therefore likely to find live insects in properly irradiated commodities. As a result, there is no independent confirmation of efficacy, such as the presence of dead insects treated by other phytosanitary methods. Therefore, the efficacy of irradiation treatments must be assured through appropriate research to confirm the process. Precise definitions of post-irradiation responses are therefore required for the benefit of government inspectors and commercial users.

4. Objectives of the First Research Coordination Meeting

The objectives of this first RCM were to:

- Discuss the plans and proposed research of individual participants.
- Facilitate a broader understanding of the relationship each participant has to the overall objectives of the CRP.
- Promote interaction between the participants.
- Prepare recommendations and guidelines to facilitate project tasks.

The intention was to provide a better understanding of the CRP objectives and for participants to agree on a common approach and way forward. The meeting recognized that research work plans and activities, and the CRP framework, may need to be refined in order to better meet the objectives of the CRP.

5. RCM Presentations

International Standardization Activities

David H. Byron, IAEA

The meeting was informed of international initiatives related to phytosanitary applications of irradiation, including activities of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, and specifically the Joint Division subprogramme on Improving Food Safety and Consumer Protection. It was noted that the subprogramme project related to Post Harvest Phytosanitary Applications of Food Irradiation to Facilitate International Trade and sought to improve Member State capabilities in the implementation of irradiation in food and agricultural commodities by:

- Providing technical input in the establishment of international standards through the International Plant Protection Convention.
- Training personnel in the application of international standards and the operation of irradiation facilities.
- Fostering information exchange through the expansion of web based resources and databases.
- Promoting research and development through coordinated research projects.
- Providing scientific and technical input to the technical cooperation programme.

It was noted that these activities were undertaken in close collaboration and cooperation with the Joint Division Insect Pest Control subprogramme so as to fully integrate the control of both pre-harvest and post-harvest pests in commodities destined for international trade.

The meeting was further informed that the IPPC was an international treaty to prevent the spread and introduction of pests of plant and plant products and to promote appropriate measures for their control, including the development of International Standards for Phytosanitary Measures under the Technical Panel on Phytosanitary Treatments (TPPT) and the Technical Panel on Fruit Flies (TPFF). It was noted that the Joint Division provided experts to both IPPC technical panels and also hosted four annual technical panel meetings at the IAEA from 2006 - 2009.

The meeting noted the importance of the IPPC, as it was recognized under the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) as the relevant international standard setting organization for the elaboration of international standards to help ensure that phytosanitary measures are not used as barriers to international trade.

The meeting was assured that the Food and Environmental Protection Subprogramme would continue to focus on the application of irradiation for phytosanitary purposes, including quarantine, through collaboration with other international organizations such as the IPPC, and would continue to seek collaborative regional centers and facilities for training in the application of international standards and the operation of irradiation facilities.

Challenges and scope for developing and Adopting Generic Irradiation Treatments

Ray Cannon, UK (Member of the IPPC Technical Panel on Phytosanitary Treatments)

Mr. Cannon outlined the work and functions of the IPPC Technical Panel on Phytosanitary Treatments (TPPT) and summarized the history of irradiation treatment submissions and their assessment by the Panel.

Seventeen original irradiation treatment submissions were evaluated by the TPPT, and of these, 13 were recommended for adoption. The TPPT did not, however, approve three of the irradiation treatments but suggested that they should be resubmitted if and when further supporting data was available, including for the fruit fly, *Anastrepha suspensa*, and the two tortricid moths, *Cryptophlebia illepidata* and *C. ombrodelta*. The panel accepted that there were valid arguments to approve a minimum absorbed dose of 70 Gy for *A. suspensa*, but the treatment was not approved because the panel could not determine the level of confidence that applied to the efficacy value. In the case of the two moths, the panel was unwilling to extrapolate from experiments carried out using artificial diet to larvae within fruits.

Mr. Cannon also summarized some of the concerns expressed by the TPPT in considering the 400 Gy generic dose treatment for Insecta (with the exception of pupae and adults of Lepidoptera). Although the TPPT recommended adoption of the generic 150 Gy irradiation treatment for fruit flies, the proposed 400 Gy generic treatment proved to be more difficult, despite additional information on the scientific rationale for this extrapolation provided by the submitter. The concerns of the Panel related to a number of issues, including:

- Extension of species data to the whole genus.
- Extending beyond the genus level to the family level.
- Extension of applicability to fruit, vegetables, nuts, flowers and foliage.
- Whether there was any evidence that contradicted the submission.
- How to deal with low numbers of insects in some studies.
- Where to set the ED for the treatment when there were a variety of different efficacies from $\geq 80.00\%$ to $\geq 99.9968\%$.

Concerns of the Panel regarding the generic irradiation schedules also related to whether existing studies have identified the most radio-tolerant species within the genus; whether the degree of variation between related, con-generic species provide support for extrapolation to all species, and; the degree of variation within different taxonomic levels.

At the Fourth Session of the IPPC Commission on Phytosanitary Measures (CPM) in Rome from 30 Mar - 3 Apr 2009, the IPPC Secretariat presented the 14 draft standards (including fruit flies) and informed the CPM that formal objections had been received from Japan and the Republic of Korea on six draft irradiation treatments for *Conotrachelus nenuphar*, *Cylas formicarius elegantulus*, *Euscepes postfasciatus*, *Grapholita molesta*, *G. molesta* under hypoxia, and *Omphisa anastomosalis*. The Korean objection related to the presence of live intact adult insects which could be introduced and released and might therefore be detected in survey traps in the importing country. Furthermore, the presence of (albeit sterile) F₁ adults was thought to be a complicating factor which could jeopardise the status of a pest free area. The formal objection by Japan concerned irradiation treatments for *Grapholita molesta* and *G. molesta* under hypoxia because the proposed irradiation treatments were not considered to be consistent with published scientific evidence and therefore, their objection recommended a revised effective absorbed dose for *G. molesta* of 232 Gy, rather than 200 Gy. These six formal objections were discussed at the IPPC Standards Committee (SC) in early May 2009, and the TPPT is currently working to progress them, with a view to address some, if not all, of the objections by November 2009.

As a result of the above discussions, the following eight irradiation treatments have been adopted and included in ISPM 28 on Phytosanitary Treatments for Regulated Pests:

Annex 1	Irradiation treatment for <i>Anastrepha ludens</i>
Annex 2	Irradiation treatment for <i>Anastrepha obliqua</i>
Annex 3	Irradiation treatment for <i>Anastrepha serpentina</i>
Annex 4	Irradiation treatment for <i>Bactrocera jarvisi</i>
Annex 5	Irradiation treatment for <i>Bactrocera tryoni</i>
Annex 6	Irradiation treatment for <i>Cydia pomonella</i>
Annex 7	Irradiation treatment for fruit flies of the family Tephritidae (generic)
Annex 8	Irradiation treatment for <i>Rhagoletis pomonella</i>

The Importance of a Common Approach to the Application and Reporting of Absorbed Radiation Dose

Andrew Parker, Entomology Unit, IAEA

Dosimetry was introduced and discussed, as was the different types of dosimeters for routine, transfer standard and reference use. The importance of accurately measuring dose and having a dosimetry system that can be traced to a recognized standard was discussed, as was the importance of recording and reporting results in a consistent manner, including uncertainty associated with the measurement. The Entomology Unit at Seibersdorf have a ⁶⁰Co irradiation unit (Gamma Cell 220) and a low energy X-ray irradiator (RS2400). The routine dosimeters are Gafchromic film and the Units Standard Operating Procedure is available on-line¹.

Dose Comparison Scheme

On behalf of the Insect Pest Control Section, Mr. Parker proposed to provide a dose comparison scheme for CRP participants. This would be free of charge, and use a set of test (non-irradiated) and control Gafchromic dosimeters (0 and 100 Gy irradiation). It was proposed to repeat the comparison exercise annually.

The meeting noted that dose inter-comparisons cannot replace calibration. The purpose of a comparison scheme is to increase confidence in reported dose, irradiation procedures and dosimetry systems but not to replace calibration. The comparison scheme would be valuable for the majority of CRP participants, especially for those where the irradiation treatment and dosimetry is performed as a service by a separate group.

¹ <http://www-naweb.iaea.org/nafa/ipc/public/ipc-gafchromic-dosimetry-sterile-insect-technique.html>

Contract Holder Presentations

Gamma Radiation Quarantine Treatments for Different Groups of Arthropods

Celina Inés Horak, Argentina

Research to establish irradiation quarantine treatments for *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), *Hemiberlesia lataniae* (Hemiptera: Diaspididae) and *Tetranychus urticae* (Acari: Tetranychidae) will be undertaken. Research will determine the minimum radiation doses required to ensure quarantine security for each species and study the effect of low oxygen content on the effectiveness of irradiation treatment with one species; *Spodoptera frugiperda*.

Source: ^{60}Co multipurpose (semi-commercial irradiation facility).

Dosimetry: National Commission of Atomic Energy. Fricke (used routinely for this research), Silver dichromate and alanine dosimeters in near future.

Parameters reported: Dose, dose rate, maximum dose ($D_{\max}$), minimum dose ($D_{\min}$), dose mapping.

Use of Ionizing Radiation to Control Eriophyes litchi (Litchi Rust Mite), Ecdytolopha aurantiana (Orange Fruit Borer) and Tuta absoluta (Tomato Worm)

Valter Arthur, Brazil

Research to determine radiation doses for *Eriophyes litchii*, (Litchi Rust Mite), *Ecdytolopha aurantiana* (Orange Fruit Borer) and *Tuta absoluta* (Tomato Worm). Insects will be reared on an artificial diet and in natural hosts; work will identify an irradiation dose for insects and mites that will prevent adult emergence or will sterilise adults, and; compare the effects of irradiation and other quarantine treatments on fruit quality.

Source: ^{60}Co (Gamma cell 220).

Dosimetry: Centre for Nuclear Energy in Agriculture, San Paulo. Gammachromic (used routinely).

Parameters reported: Dose, dose rate, time.

Irradiation as a Quarantine Treatment of Orange Against Citrus Mites

Meiying Hu, China

Research to develop a large scale rearing method for citrus rust mite (*Phyllocoptruta oleivora* Ashmead) and citrus red mite (*Panonychus citri* McGregor) will be undertaken, and the irradiation dose suitable for quarantine purposes determined. The combined effect of extended cold storage following irradiation treatment will be investigated. Large scale tests will also investigate irradiation treatment of Shantang mandarin in different packaging materials. Analysis of the quality and shelf-life of Shantang mandarin post irradiation and cold storage will be undertaken. This study could also be expanded to investigate other mites (e.g. six-spotted mite and citrus yellow mite).

Source: ^{60}Co Gamma cell at South China Agricultural University.

Dosimetry: Fricke (used routinely).

Parameters reported: Dose, dose rate, time.

Irradiation as a Phytosanitary Treatment for Controlling *Carposina sasakii* (Matsumura)

Guoping Zhan, China

A rearing method will be established in the laboratory using a range of environmental conditions. The minimum effective dose for phytosanitary irradiation of the most suitable tolerant development stage will be estimated and confirmed based on statistical analysis and a large scale test. The effects of different dose rates and of gamma and X-ray irradiation on the larvae will be investigated. The radio-tolerance of the treated fruits will be evaluated.

Source: x-ray irradiation provided by NUCTECH Company Limited; gamma rays (Cobalt-60) provided by Institute of Agro-food Science and Technology, Chinese Academy of Agriculture Science.

Dosimetry: Fricke (used routinely).

Parameters reported: Dose, dose rate, time.

Evaluating Gamma Irradiation as a Post-Harvest Treatment for the Control of Citrus Mealybug on Citrus in South Africa,

Hendrik Hofmeyr, South Africa

The research aims to establish and rear citrus mealybugs (*Planococcus citri*) and conduct dose response studies on the various life stages. Adult mealybugs will be irradiated to several different doses and stored at 25°C. Crawler production and adult survival will be evaluated by comparison with non-irradiated controls. The lowest most effective dose treatment will be determined using mealybugs of all ages. The lowest gamma irradiation dose resulting in complete control of at least 30 000 citrus mealybugs will be determined for either mortality or prevention of further progeny production.

Source: ⁶⁰Co (Panoramic irradiator).

Dosimetry: iThemba LABS (Laboratories for Accelerator Based Sciences). Traceability to national standard (C.S.I.R: Council for Industrial and Scientific Research). Fricke (used routinely).

Parameters reported: Source strength, initial calibration and details of dosimetry system, uncertainty level, confidence interval, dose mapping, measured absorbed dose, D_{min}, D_{max}, dose rate (time of exposure), dose uniformity, temperature.

Irradiation as a Phytosanitary Measure for Grape Vine Moth, (*Lobesia botrana*) and Khapra beetle (*Trogoderma granarium*)

Mohammed Mansour, Syrian Arab Republic

The objectives of this research are to study the radio-sensitivity of the grape moth and Khapra beetle, establish the quarantine dose based on the criteria acceptable to regulatory authorities, undertake a pilot scale study of the tolerance of commodities to irradiation treatment, and establish the optimum conditions for treatment.

Source: ⁶⁰Co gamma cell (and a semi-commercial ⁶⁰Co irradiation facility available for confirmatory tests)

Dosimetry: Syrian Atomic Energy Commission. Fricke (used for research) dichromate, alanine.

Parameters reported: Dose rate and dose.

Generic Irradiation Dose to Provide Quarantine Security for Agromyzid Leafminers

Berna Ozyardimci, Turkey

This research will involve *Liriomyza bryoniae*; *L. huidobrensis*; *L. trifolii* and *L. sativa* (Diptera: Agromyzidae). The dose range required to ensure quarantine security will be identified in preliminary range-finding tests. Confirmatory tests of up to 30,000 insects will then be undertaken. Chemical analysis and

sensory evaluation of irradiated pea will also be undertaken. Depending on results obtained, the aim of this research is to develop a generic treatment for Agromyzid leafminers in the most tolerant species.

Source: ^{60}Co Gammacell PXGamma 30 issued by the Hungarian Atomic Energy Authority (Hungary).

Dosimetry: Turkish Atomic Energy Authority and National Standards Laboratories of Turkey. Fricke, Alanine, gammachromic (routine).

Parameters reported: (Saraykoy Nuclear Research and training Centre) Dose rate, $D_{\min}$, $D_{\max}$, temp, dose maps, and humidity.

Effects of Gamma Irradiation at Quarantine Doses on Three Species of Mealybugs (Hemiptera: Pseudococcidae) Infesting Red Dragon Fruits

Thi The Doan, Vietnam

This research seeks to establish the minimum radiation doses required to ensure quarantine security against mealybugs on Red Dragon fruit. The study will include investigating the effects of gamma irradiation on three species of mealybugs (*Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *P. minor*) (Hemiptera: Pseudococcidae). The dose required to prevent larvae from developing into adults and to prevent adults from reproducing will be established by large scale confirmatory tests on the most radio-tolerant stage of the three species.

Source: ^{60}Co commercial scale gamma unit.

Dosimetry: Research and Development Centre for Radiation Technology, Vietnam. Fricke (for this research), AgCr_2O_7 .

Parameters reported: Dose rate, time, dose, product density and will provide dose map if necessary.

Generic Dose of Gamma Irradiation for Quarantine Treatment of Mangosteen Insect Pests

Achmad Nasroh Kuswadi, Indonesia

Colonies of mealybugs (*Pseudococcus* sp.) and thrips (*Scirtothrips* sp.) will be established. The bionomy of the mealybug and thrips will be investigated to obtain data on the life cycle. The dose range required to ensure quarantine security will be established.

Source: Three ^{60}Co irradiators (i) Gammacell for this research, (ii) Gamma Chamber and (iii) Multipurpose Panoramic Irradiator can be used for large scale confirmatory tests.

Dosimetry: Centre for Application of Isotopic Radiation Technology (Indonesian), Fricke.

Reported parameters: Dose, dose rate and time.

Development of Generic Irradiation Doses for Phytosanitary Treatment of Mealybug Spp. Infesting Agricultural Commodities

Ranjana Seth, India

A mass rearing facility for *Paracoccus marginatus*, *Phenacoccus solenopsis* and *Maconellicoccus hirsutus* will be established and run an asynchronous culture. Studies on the biocharacteristics of each species will be undertaken and the effects of irradiation will be studied. The criteria for determining efficacy of irradiation as a quarantine treatment both in isolation and in infested commodities will be identified.

Source: ^{60}Co , Gamma cell 5000 (Institute of Nuclear Medicine and Allied Sciences).

Dosimetry: BRIT section of BARC (Bhabha Atomic Research Centre), Fricke.

Parameters reported: Dose rate, time and dose.

Agreement Holder Presentations

Development of Generic Irradiation Doses for Quarantine Treatments

Peter Leach, Australia

Irradiation research undertaken at the Australian Nuclear Sciences and Technology Organisation (ANSTO) is capable of treating large volumes of fruit in commercial packaging (e.g. 8 x 7.5 kg cartons at a time). Treatments are applied using fractionated doses to obtain dose uniformity ratios of close to 1.0 (ratio of $D_{\max}/D_{\min}$). Routine dosimeters (Fricke) are calibrated in a cobalt 60 radiation field in which the dose rate has been determined using reference dosimeter measurements. The dosimetry is traceable to the Australian and United Kingdom standards for absorbed dose.

Source: ^{60}Co Gamma Technology Research Irradiator.

Dosimetry: Australian Nuclear Science and Technology Organisation (ANSTO), Fricke (routinely used).

Parameters reported: Dose range, confidence interval, dose rate, duration of exposure, temperature.

Exploring the Effects of Controlled Atmospheres on Insect Stress Physiology and the Efficacy of Irradiation Treatments for Insect Control

Daniel Hahn, USA

Whether irradiation in hypoxic conditions reduces cellular oxidative damage in the Caribbean fruit fly, *Anastrepha suspensa* will be investigated (this pest has previously been shown to have a better post irradiation survival and performance when irradiated in hypoxia). The magnitude and duration of cellular oxidative stress mechanisms induced by hypoxic conditions will be investigated, and the relationship with pest survival and performance following irradiation will be determined at oxygen levels applicable to commodity storage. The initial approach is to quantify oxidative damage to DNA, RNA, membrane lipids, and proteins in Caribbean fruit flies irradiated to a range of doses under low and normal oxygen conditions.

Source: ^{137}Cs irradiation unit, E-Beam and X-ray (research uses ^{137}Cs and E-beam). Florida Department of Agriculture.

Dosimetry: Performed by researcher, Gafchromic film.

Parameters reported: Dose, dose rate.

Development of Generic Irradiation Doses for Quarantine Treatments

Guy Hallman, USA

Insects from three families representative of quarantine pests and for which little information of radiotolerance is available will be studied. Eggs and larvae of *Heliothis virescens* may be found on shipped commodities making the last instar larva the most tolerant stage. The measure of efficacy will be prevention of adult emergence. Although *Liriomyza trifolii* tends to leave the plant to pupate, pupae may be found on foliage, so the most tolerant stage that could be present on shipped commodity is the late pupal stage. Doses to stop adult emergence from late pupae are generally too high to be used as phytosanitary treatments so a later stage of development will be the measure of efficacy. With the Asian citrus psyllid, *Diaphorina citri*, all stages may be present on shipped commodity so the adult is the most radiotolerant stage. The measure of efficacy will be prevention of reproduction to the greatest degree feasible, which could be prevention of oviposition or hatch of F_1 eggs or subsequent development beyond the first instar.

Source ^{137}Cs (Husman). US Animal and Plant Health Inspection Service (APHIS).

Dosimetry: Performed by researcher, Gafchromic, Opti-Chromic.

Parameters: include dose, $D_{\max}$ and $D_{\min}$.

Development of Generic Irradiation Doses for Quarantine Treatment Using E-Beam, X-Rays & Gamma

Suresh Pillai, USA

The National Center for E-Beam Research at Texas A&M University has two 10 MeV E-Beam LINACs (linear accelerators), and one 5MeV X-ray LINAC. A full-scale dosimetry system utilizing state of the art NIST traceable alanine dosimeters and Bruker E-Scan EP spectroscopy is available. Additionally, the Center will have access to a Faxitron Cabinet x-ray system with output voltage of 10-110kV. Texas A&M University also operates a 1 megawatt TRIGA “swimming pool reactor” which can be used to generate gamma rays from Lanthanum-140.

The insects to be used on these studies will be the western flower thrips, *Frankliniella occidentalis*, the fall armyworm, *Spodoptera frugiperda* and the serpentine leafminer, *Liriomyza trifolii*. The equipment and facilities that are available at Texas A&M University are unique in that we can compare irradiation effects using 3 different sources namely 10 MeV E-Beam, 5 MeV X-ray, and Lanthanum-140 derived gamma. Therefore we have the ability to perform studies using different dose rates but with the same dosimetry system, the alanine system.

Source: X-ray, Gamma (^{140}La) and E-Beam, National E-Beam Center.

Dosimetry: performed by researcher, alanine pellets (for this research), Gammachrome film.

Parameters: include dose, dose rate, scan rate, beam power.

6. Conclusions and Recommendations of the First RCM

Conclusions

1. The meeting noted that the proposed work is an ambitious effort that addresses 29 species from 13 arthropod families, including several quarantine pest families for which no data are currently available (Annex D).
2. The proposed research will enable the development of generic and specific doses for pests and pest groups of quarantine importance, thereby reducing technical barriers and facilitating international trade in agricultural produce.
3. The meeting recognised that it is necessary to investigate factors beyond absorbed dose that may affect efficacy and which have not been previously addressed.
4. In recognition of the importance of common procedures in research and dosimetry, a research protocol was developed (Annex C).
5. Representation from the Technical Panel on Phytosanitary Treatments (TPPT) was critical in providing valuable feedback on IPPC operations and in facilitating the consideration of irradiation treatments in the development of further ISPM standards.
6. Increasing utilisation of irradiation technology will require further training of quarantine inspectors and regulatory authorities.

Recommendations

1. The meeting strongly recommended continued close collaboration and cooperation with the TPPT in order to address issues raised by Member States and to further facilitate the application of irradiation for phytosanitary purposes, including the development of international standards.
2. Immediate and continuing liaison with the IPPC Secretariat is necessary to facilitate the adoption of the 6 withheld irradiation treatments and to coordinate the submission of the additional irradiation treatments developed under this coordinated research project.

3. The use of the IDIDAS (International Database on Insect Disinfestation and Sterilisation) database to collate published information relating to irradiation treatments of pests is strongly encouraged.
4. It was strongly recommended that Agreement holders should collaborate and assist contract holders to facilitate the efficient and thorough generation of data, especially between participants studying similar pests.
5. The meeting encouraged international organization funding and implementation of training courses related to the application of international standards, the operation of irradiation facilities and quarantine inspection and regulatory control.

7. Agreed Action Plan and Logical Framework

Action Plan (Activities)

Activity	2009	2010	2011	2012	2013
Advertise the CRP (December 2008). Receipt of research contract and agreement proposals. Award contracts and sign agreements by end April 2009.	X				
Organise 1 st RCM (July/August 2009) to discuss overall CRP work plan, agree on research protocols, governance, quality assurance, record keeping and reporting.	X				
Award Research Contracts	X		X		
Phase 1: 18 months work programme	X	X			
Organise 2 nd RCM in March/April 2011 to review the work conducted in Phase 1 based on progress reports and presentations. Develop the detailed work plan for Phase 2 and ensure that the CRP objectives are met.			X		
Phase 2 : Work programme			X	X	
Organise 3 rd RCM (Oct 2012) to review work conducted in Phase 2 and agree final phase 3 work programme.				X	
Phase 3: Work programme				X	X
Final RCM: Late 2013 Review Phase 3 work. Prepare a TECDOC and /or research papers to an appropriate journal.					X

Logical Framework

	Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
Overall Objective	Enhance opportunities for international trade in foods of plant origin subject to insect infestation	Decrease in detained/rejected consignments after inspections	Reports to national authorities and regional plant protection organisations	Commitment by all participating partners to report on national data
Specific Objective	Provide data to develop generic irradiation doses for quarantine treatments for consideration in providing an appropriate level of assurance against insect pest incursions	Monitoring programmes for surveillance of quarantine pests	Reports to national authorities and regional plant protection organisations	Commitment by all participating partners to report on national data
Outcomes	Generic doses for a range of quarantine pests/commodities adopted by Commission on Phytosanitary Measures	Data and including dose response information published and provided to IPPC Technical Panel on Phytosanitary Measures	Laboratory results and published reports	Acceptance of recommended generic doses by IPPC Member States
Outputs	Validated generic doses/commodities and protocols recognised, harmonised SOPs	Protocols and SOPs produced	Reports submitted to the IPPC, IAEA, FAO and national authorities	Continued commitment by all partners
Activities	Consultants meeting	Consultants Meeting report	Meeting report and recommendations	Consultants identified, available and meeting held
	Research contract and agreement holders identified; contracts and agreement signed	First RCM	Meeting report and recommendations	Continued commitment by all parties
	Work programme to develop and validate dosimetry and research methodology	1 st , 2 nd and 3 rd RCM	Meeting reports	Continued commitment by all parties
	Quality check protocols for generic doses	3 rd RCM	Meeting reports	Continued commitment by all parties
	Prepare SOPs, scientific papers and TECDOC.	Final RCM	Meeting reports, TECDOC, SOPs and papers published	Continued commitment by all parties

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First Research Coordination Meeting
5 – 9 October 2009
The Development of Generic Irradiation Doses for Quarantine Treatments

IAEA Vienna, Austria
Room A0742

Scientific secretary: Carl Blackburn (FEP/NAFA) c.blackburn@iaea.org
Administrative secretary: Stella Attakpah (FEP/NAFA) s.attakpah@iaea.org

Agenda

Day 1: Monday, 5 October 2009		
08:00	Registration (UN Pass Office, Gate 1)	All
09:00	Welcome & Opening Address	Qu Liang, IAEA
09:15	Introductions & Presentation of Participants	David Byron, IAEA
09:30	Election of Chairperson	David Byron, IAEA
09:40	Adoption of Agenda	Chairman
09:50	Meeting Objectives	Carl Blackburn, IAEA
10:00	CRP Background	Carl Blackburn, IAEA
<i>10:30</i>	<i>Break</i>	
11:00	International Standardisation Activities	David Byron, IAEA
11:30	Challenges and Scope for Developing and Adopting Generic Irradiation Treatments	Ray Cannon, UK
12:00	The Importance of a Common Approach to the Application and Reporting of Absorbed Radiation Dose	Andrew Parker, IAEA
<i>12:30</i>	<i>Lunch / Administrative Arrangements</i>	
<i>Session 1: Contract Holder Presentations</i> (Proposed Research work plan, summary of work to date and meeting the objectives of the CRP)		
14:00	Gamma Radiation Quarantine Treatments for Different Groups of Arthropods	Celina Inés Horak, Argentina
14:45	Use of Ionizing Radiation to Control <i>Eriophyes litchi</i> (Litchi Rust Mite), <i>Ecdytolopha aurantiana</i> (Orange Fruit Borer) and <i>Tuta absoluta</i> (Tomato Worm)	Valter Arthur, Brazil
<i>15:30</i>	<i>Break</i>	
16:00	Irradiation as a Quarantine Treatment of Orange Against Citrus Mites	Hu Meiyong, China

16:45	Irradiation as a Phytosanitary Treatment for Controlling <i>Carposina sasakii</i> (Matsumura)	Guoping Zhan, China
17:30	End of day 1	

Day 2: Tuesday, 6 October 2009

Session 1 (Continued): Contract Holder Presentations (Proposed Research work plan, summary of work to date and meeting the objectives of the CRP)

08:30	Evaluating Gamma Irradiation as a Post-Harvest Treatment for the Control of Citrus Mealybug on Citrus in South Africa	Hendrik Hofmeyr, South Africa
09:15	Irradiation as a Phytosanitary Measure for Grapes Infested with the Grape Vine Moth, <i>Lobesia botrana</i>	Mohammed Mansour, Syrian Arab Republic
10:00	Break	
10:30	Generic Irradiation Doses to Providing Quarantine Security for Bruchid Seed Weevils and Agromyzid Leafminers	Berna Ozyardimci, Turkey
11:15	Effects of Gamma Irradiation at Quarantine Doses on Three Species of Mealybugs (<i>Hemiptera: pseudococcidae</i>) Infesting Red Dragon Fruits	Thi The Doan, Vietnam
12:00	Lunch	
13:00	Generic Dose of Gamma Irradiation for Quarantine Treatment of Mangosteen Insect Pests	Achmad Nasroh Kuswadi, Indonesia
13:45	Development of Generic Irradiation Doses for Phytosanitary Treatment of Mealybug Spp. Infesting Agricultural Commodities	Ranjana Seth, India

Session 2: Presentations from Agreement Holders

14:30	Development of Generic Irradiation Doses for Quarantine Treatments	Peter Leach, Australia
15:15	Break	
15:45	Exploring the Effects of Controlled Atmospheres on Insect Stress Physiology and the Efficacy of Irradiation Treatments for Insect Control	Daniel Hahn, USA
16:30	Development of Generic Irradiation Doses for Quarantine Treatments	Guy Hallman, USA
17:15	Development of Generic Irradiation Doses for Quarantine Treatment Using E-Beam, X-Rays and Gamma	Suresh Pillai, USA
18:00	Early evening - Reception / Meal (venue to be arranged)	All

Day 3: Wednesday, 7 October 2009		
Session 3: Discussion and production of protocols and guidelines		
08:30	Conclusions and Recommendations from the Consultants Meeting	Carl Blackburn, IAEA
09:00	Discussion / drafting • Research protocols and guidelines to ensure consistency (a) The application and reporting of dosimetry (b) Definitions of the measure of efficacy (c) Other • Governance • Quality assurance • Record keeping • Reporting	
Visit to the Agency's Laboratories at Seibersdorf		
The Agency's Laboratories are located in the vicinity of the village of Seibersdorf, 35 km southeast of Vienna, at the premises of the Austrian Research Center.		
11:30	Bus to Seibersdorf	
12:30	<i>Lunch at Seibersdorf</i>	
14:00	Visit Seibersdorf	
16:00	Return to Vienna International Center (VIC)	
17:00	<i>End of day 3</i>	

Day 4: Thursday, 8 October 2009		
Session 3 (continued): Production and agreement of protocols and guidelines		
08:30	Producing agreed guidelines on the application and reporting of dosimetry to ensure consistency	All
09:30	Agreement on common / standard research protocols (e.g. definitions of the measure of efficacy for irradiation as a phytosanitary option for all the arthropod groups studied under the CRP)	All
10:30	<i>Break</i>	
Session 4: Preparation of final draft report (Discussion of a common approach and way forward to achieve the CRP objectives)		
11:00	Session on country work plans (Summary of country work plans and contributions to overall CRP objectives)	All
13:00	<i>Lunch</i>	
14:00	Session on draft conclusions and recommendations (Production of draft conclusions and recommendations to meet the CRP objectives)	All
15:30	<i>Break</i>	
16:00	Review and summary - Protocols and guidelines - Country work plans and the CRP objectives - Conclusions and recommendations	All
17:30	<i>End of day 4</i>	

Day 5: Friday, 9 October 2009		
Session 5: Preparation of final report		
08:30	Drafting session on final report	All
10:30	<i>Break</i>	
11:00	Drafting session on final report	All
12:30	<i>Lunch</i>	
13:30	Identifying project strengths, weaknesses and opportunities	All
14:00	Presentation of final meeting report	
15:30	<i>Break</i>	
16:00	Discussion and adoption of the report, including conclusions and recommendations	All
16:30	Closing remarks	

RESEARCH PROTOCOL

1. Research materials

To perform treatment research to control quarantine pests it is necessary to know its basic biology as well as define how the pests used in the research will be obtained. The experiments should be carried out on the commodity infested naturally in the field and/or with laboratory-reared pests that are used to infest the commodity preferably in a natural form. The method of rearing and feeding should be carefully detailed.

Where plant material is used during irradiation trials it should be of a suitable quality. If studies are done with pests in an artificial circumstance, preliminary testing must be undertaken to demonstrate that at least an equal absorbed dose is required to achieve the same control with the pest as in the normal situation. Where the artificial circumstance provides a more radio-tolerant situation, the derived dose for an effective treatment will be an over-estimate but could still be acceptable.

It is recommended to archive voucher specimens, for example in a reference collection, of the appropriate developmental stages of the pests studied in order to, among other reasons, resolve possible future disputes on identification.

2. Dosimetry

The dosimetry system should be calibrated, certified and used according to recognized international standards. The minimum and maximum doses absorbed by the irradiated product should be determined, striving for dose uniformity. Routine dosimetry should be conducted, and a dosimetry report should be provided for each experiment.

The IAEA/FAO laboratory at Seibersdorf will provide a periodic dose comparison service. Participation is recommended.

International Standards and other guides are available to assist with conducting dosimetry for research on food and agricultural products, including:

ISO/ASTM 51261 Standard Guide for Selection and Calibration of Dosimetry Systems for Radiation Processing.

ASTM F1355-06 Standard Guide for Irradiation of Fresh Agricultural Produce as a Phytosanitary Treatment.

ISO/ASTM 51900 Standard Guide for Dosimetry in Radiation Research on Food and Agricultural Products.

Gafchromic® Dosimetry System for Sterile Insect Technique (SIT)².

The maximum dose measured during the confirmatory part of the research will be the minimum dose required for the approved treatment. It is recommended to keep the maximum-minimum dose ratio as low as possible.

The key parameters required for dosimetry and reporting dosimetry include the following:

1. Calibration of radiation field inside the chamber with confidence interval and traceability to a recognized national standard.
2. A statement or reference to details of the dosimetry system employed.

² <http://www-naweb.iaea.org/nafa/ipc/public/ipc-gafchromic-dosimetry-sterile-insect-technique.html>

3. Uncertainty / confidence interval on the dosimetry system.
4. Dose mapping exercise for each configuration used ($D_{\max}$, $D_{\min}$, and dose distribution). The loading pattern for dose mapping should be recorded (a diagram for example).
5. The loading pattern for subsequent treatments should be the same as that used in the dose mapping exercise and should be recorded with reference to the dose mapping exercise.
6. The location for the placement of routine dosimeter(s) and the relationship between the dose received by dosimeter(s) at the routine location and the $D_{\max}$ and $D_{\min}$ (obtained from dose mapping).
7. Source strength.
8. Irradiation Certificate with the following information:
 - Target dose.
 - Measured dose (and confidence interval).
 - Dose rate.
 - Dose uniformity ratio.
 - A statement of whether the dose was delivered in a single treatment or by multiple exposures (for example by so called “dose fractionation” or as part of the normal operation of the irradiation unit).

3. Estimation and confirmation of minimum absorbed dose for treatment

3.1 Defining the treatment end-point

The required response should be described as precisely as feasible. It is necessary to define the developmental control point accurately.

Acute mortality is rarely, if ever, the objective of irradiation for phytosanitary purposes. Inability to reproduce may be an appropriate response for pest(s) that are not vectors and remain on or in the commodity. The response required depends on the pest and its biological characteristics.

The critical developmental stage on which the efficacy of the treatment is based must be precisely defined. A range of specific treatment outcomes may be stated where the required response is the inability of the pest to reproduce. These may include:

- Prevention of adult emergence.
- Prevention of egg laying.
- Prevention of development of phanerocephalic pupa.
- F_1 eggs do not hatch.

3.2 Preliminary tests

The following steps should be carried out to estimate the dose required to ensure quarantine security:

- Radiosensitivity of the most radio-tolerant stage that can be present on the commodity as shipped must be established, even if it is not the most commonly found stage in the commodity. Note that in general radiotolerance increases with increasing development stage.
- The minimum absorbed dose will be determined experimentally. Where pertinent data do not already exist, one approach is to use several dose levels and a control for the appropriate developmental stage (appropriate to commodity as shipped), with a sufficient number of individuals for each of the doses and a number of replicates. Another approach is to employ a dose that will probably not be fully effective, and increase the dose for the next experimental treatment, based on the results found, as part of an iterative series of experiments to collect data on dose efficacy with increasing absorbed dose.

- Information on the life cycle of the pest should be clearly documented. The number of pests present and the life-stages present at the time of treatment should be recorded for each trial. This information is essential in order to determine the treatment efficacy.

- During the period of post-treatment observation of commodities and associated pests, both the treated and control must remain under favorable conditions for pest survival, development, and reproduction so that the appropriate parameters can be measured. The untreated control must develop and/or reproduce normally for a given replicate of the experiment to be valid. A review of the experimental methodology should be undertaken if the response of the pests in the untreated control is unusual (e.g. an unusually high mortality).

When studying several closely related pest species it may be useful to carry out preliminary testing to determine the most radio-tolerant species (taking into account life stages). The most tolerant species can be used to establish an effective treatment for the whole group.

3.3 Large scale (confirmatory) tests

To confirm the validity of the estimated minimum dose required to provide quarantine security, it is necessary to treat a large number of individuals of the organism while achieving the desired result, be it prevention of pest development or inability to reproduce offspring. The number treated will depend on the required level of confidence. The level of efficacy of the treatment should be established and be technically justifiable.

Records and data should be retained and made available to interested parties for consideration in establishing an agreed commodity treatment.

Annex D

Taxonomic groups represented in CRP on the development of generic irradiation doses for quarantine treatments

Order	Insects Coleoptera Dermestidae	Mites Eriophyidae	Mites Tetranychidae	Insects Diptera Agromyzidae	Insects Hemiptera Aleyrodidae	Insects Hemiptera Diaspididae	Insects Hemiptera Pseudococcidae	Insects Hemiptera Psyllidae	Insects Lepidoptera Carposinidae	Insects Lepidoptera Gelechiidae	Insects Lepidoptera Noctuidae	Insects Lepidoptera Tortricidae	Insects Thysanoptera Thripidae
Argentina			1		1	1					1		
Brazil		1							1			1	
China2		1	1										
Vietnam							3						
Turkey				4									
China1								1					
Syria	1											1	
Indonesia							1						1
India							3						
USA1				1				1			1		
S Africa							1					1	
USA2				1							1		1
Total	1	2	2	6 ^A [4 species]	1	1	8	1	1	1	3 ^B [2 species]	3	2

^A Three researchers propose to study the same agromyzid; therefore four different species proposed.

^B Two researchers (Argentina and USA2) propose to study the same noctuid; therefore two different species proposed.