

Collaboration between monitoring authorities, regulatory authorities and test facilities on GLP principles provides confidence in data quality with an emphasis on sound science. GLP: an example of a focused effort that paid off

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Summary. The herbicide atrazine has been the subject of numerous studies investigating its potential effects on amphibians. The United States Environmental Protection Agency (EPA) required the atrazine registrant to conduct a tiered study approach. Tier I of the studies involved laboratory studies to determine whether atrazine affects amphibian gonadal development. Several good laboratory practice (GLP) inspections were conducted during the Tier I atrazine amphibian study entitled “Response of larval *Xenopus laevis* to atrazine exposure: assessment of metamorphosis and gonadal morphology”. These inspections were conducted on each of the in-life (Phase I) test facilities (TF), *i.e.*, Wildlife International (WLI) Ltd. (Easton, Md, USA) and the Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB) (Berlin, Germany). All of the inspections were conducted in conjunction with the EPA GLP monitoring authority (MA), the Office of Enforcement, Compliance and Assurance (OECA) as well as auditors from the regulatory authority (RA) Office of Pesticide Programs (OPP). The inspection of the German facility also included representatives of the German equivalent of OECA. In Phase II of the Tier I study, tissue samples collected by both IGB and WLI during Phase I were prepared for histology and reviewed by a veterinary pathologist at the Experimental Pathology Laboratory (Vienna, Virginia, USA). The cooperation between the MA, RA and the TF allowed OPP to ensure the GLP principles were being followed as well as allowing everyone involved to bring up some higher level science issues associated with the study execution.

Key words: good laboratory practice, test facilities, atrazine, inspections.

Riassunto (*Collaborazione sui principi di BPL tra autorità di monitoraggio, autorità regolatorie e centri di saggio: un esempio di impegno preciso di piena soddisfazione*). L'erbicida atrazina è stato oggetto di numerosi studi tesi ad appurare i suoi effetti negativi sulle specie anfibe. La Environmental Protection Agency (EPA) degli Stati Uniti aveva richiesto, per la registrazione dell'atrazina, di condurre studi sequenziali. La Fase I di tali studi, che ha coinvolto più laboratori, ha avuto per scopo l'esame dei possibili effetti negativi dell'atrazina sullo sviluppo delle gonadi della rana africana. Durante questa fase dello studio denominato “Risposta dello *Xenopus laevis* allo stato larvale alla esposizione all'atrazina: accertamento della metamorfosi e della morfologia delle gonadi”, sono state effettuate diverse audizioni di buona pratica di laboratorio (BPL). Queste audizioni sono state condotte in ciascuno dei Centri di Saggio (CdS) coinvolti nella Fase I *in vivo*, e cioè la Wildlife International (WLI) Ltd. (Easton, Md, USA) ed il Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB) (Berlino, Germania). Tutte le audizioni sono state fatte congiuntamente con l'autorità di monitoraggio (MA) dell'EPA per la BPL, l'Ufficio per il Controllo, la Conformità e l'Assicurazione (UCCA) e gli uditori dell'Ufficio per il Programma Antiparassitari (UPA) della autorità regolatoria (AR). L'audizione del CdS tedesco ha coinvolto anche esponenti dell'equivalente in Germania dell'UCCA. Nella Fase II dello studio sequenziale i campioni di tessuto raccolti sia dall'IGB che dalla WLI sono stati preparati per l'esame istologico ed esaminati da un veterinario patologo dell'Experimental Pathology Laboratory (Vienna, Virginia, USA). La collaborazione tra MA, RA e CdS ha permesso all'UPA di verificare l'effettivo rispetto dei principi di BPL, nonché a tutti i partecipanti di discutere alcuni aspetti di particolare valore scientifico concernenti lo studio in oggetto.

Parole chiave: buona pratica di laboratorio, centri di saggio, atrazina, ispezioni.

INTRODUCTION

In order for a pesticide to be used legally in the United States, it must be registered. The Office of Pesticide Programs (OPP) within the US Environmental Protection Agency (US EPA) has the statutory authority to register all pesticides for use in the United States. The agency receives its authority to regulate pesticides through the Federal Insecticide Fungicide Act (FIFRA) and the Food Drug and Cosmetic Act (FDCA). As part of the registration process, specific data must be submitted by pesticide manufacturers (registrants) to support the registration decision; regulations and guidelines on data requirements for registration of pesticides are codified in the US Code of Federal Regulations 40 (CFR) Part 158 [1]. Additionally, guideline studies conducted in support of registration must be conducted in compliance with the standards of good laboratory practice (GLP) described in the 40 CFR Part 160 [2].

THE EPA PROGRAM

Over the past 30 years, US EPA has established a highly regarded program for evaluating pesticide safety. The agency's approach to decision-making is widely considered to be a model for transparency and objectivity. Regulatory decisions are intended to be consistent with available scientific information and protective of public health and the environment.

During the registration process, US EPA reviews scientific data from registrant-submitted and open literature sources. Although both open literature and registrant-submitted data are thoroughly reviewed, guideline studies required under FIFRA receive the greatest scrutiny since these studies are conducted under highly prescribed conditions. In reviewing these studies, the Agency first determines whether the results are scientifically sound. During the review process, US EPA evaluates whether the studies are consistent with its published guidelines describing each of the studies and with the GLP regulations that describe procedures to ensure high quality data from laboratory studies.

As part of the GLP process, US EPA also has a laboratory monitoring authority (MA) through US EPA's Office of Enforcement and Compliance Assurance (OECA) program that monitors testing facilities for GLP compliance. If any uncertainties are identified about the integrity of a study during the review process, the Agency can trace the original data back to their source if necessary and inspect laboratory notebooks at the test facility where the data were generated.

In addition to the established process that allows the regulatory authority (RA), *i.e.*, the OPP, to request inspections by the MA (OECA), of a test facility when data quality of a submitted study is

in question, there have also been special requests. These requests from OPP for monitoring of studies have resulted because the studies are key to its scientific assessments and may involve protocols not represented by current guidelines.

As an example, the herbicide atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,3,6-triazine; CAS 1912-24-9) is one of the most studied pesticides in the marketplace. The OPP is actively reviewing atrazine in its preregistration program and there are nearly 6 000 studies in US EPA files on the human health and environmental effects of atrazine. The Agency used these data to produce its preliminary risk assessment of atrazine in 2001, and after considering comments and additional data, the Agency issued a revised risk assessment for comment in 2002. The OPP released its regulatory position (Interim Reregistration Eligibility Decision) in January 2003 [3] which was revised in October 2003 [4] to reflect new data. However, the question of whether atrazine affects amphibian gonadal development remained uncertain and was a source of controversy. Although OPP evaluated 17 different laboratory and field studies, reporting that atrazine causes a mixture of male and female gonadal tissue in a single animal (intersex) it was determined through a rigorous assessment process and in consultation with an independent group of experts (FIFRA Scientific Advisory Panel) external to the Agency that all of the available studies contained significant uncertainties that limited the utility of the data in ecological risk assessment. Therefore no firm conclusions could be drawn about whether atrazine affects amphibian gonadal development and if so, at what exposure concentration. None of the laboratory studies were conducted in accordance with the standard aquatic toxicity protocols developed by the American Society for Testing and Materials (ASTM) International (http://www.astm.org/ABOUT/index.html#_1) twenty years ago, and each of the studies contained major uncertainties that rendered them unsound.

To ensure that the agency reaches scientifically defensible and transparent conclusions, study reviews and risk assessments undergo both internal and external scientific peer review. When the agency's review process encounters a significant scientific uncertainty that serves as a source of controversy, OPP turns to the FIFRA Scientific Advisory Panel (SAP) for independent, external, expert scientific peer review. The SAP is a federal advisory committee and as such must comply with requirements for balance, objectivity, openness, and transparency.

To resolve questions concerning atrazine's potential effects on amphibian gonadal development, the FIFRA SAP concurred [5] with the agency's proposal to require new research following a tiered process. The details of the agency's proposal can be found in the document entitled

White paper on the potential effects of atrazine on amphibians [6]. Because of the uncertainty surrounding the potential effects of atrazine on the survival, growth, metamorphosis and gonadal development in African clawed frogs (*Xenopus laevis*) [6] and other species of amphibians, in November the agency issued a data call-in (DCI) notice to Syngenta Crop Protection, Inc., and other atrazine registrants to conduct a tiered study examining the potential effects of atrazine on *X. laevis*. Consistent with the 2003 White Paper [6], the first tier (Tier 1) involved laboratory studies to determine whether atrazine affects amphibian gonadal development. In response to the DCI, the registrant developed a GLP-compliant study protocol which OPP reviewed prior to the initiation of the studies [2].

The Environmental Fate and Effects Division of the OPP in conjunction with OECA conducted on-site inspections of the Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB) and Wildlife International Limited (WIL), which were under contract to Syngenta Crop Protection, to complete the in-life portion (Phase 1) of the atrazine Tier 1 amphibian studies.

The inspections were conducted to insure that the proposed studies of atrazine's potential effect on amphibian gonadal development would satisfactorily address uncertainties identified in previous studies and that the laboratories' processes were consistent with good laboratory practice requirements [2]. IGB in Berlin, Germany, was inspected between June 26 and July 2, 2005, and WIL in Easton, Maryland, was inspected between July 11 and July 14, 2004. John Helm (OECA) focused primarily on GLP compliance while Thomas Steeger (OPP) primarily focused on the technical aspects of the studies. Stephanie Irene (OPP) participated in the inspection of WIL and focused on the technical aspects of the studies. The inspection of the German facility also included representatives of the German equivalent of OECA. Each of the studies was divided into similar phases. Phase I represented the in-life exposure portion of the study while Phase II represented the histological analysis of tissue samples collected during Phase I. In Phase II of the Tier 1 study, tissue samples collected (by both IGB and WIL) during Phase I were prepared for histology and reviewed by a veterinary pathologist at the Experimental Pathology Laboratory (EPL), Vienna, Virginia, USA. The inspection of EPL included personnel from OPP, OECA and US EPA's Office of Research and Development (ORD).

The inspections were intended to identify potential methodological issues in advance so that they could be addressed and better insure that the study would meet desired objectives. The inspections helped to foster a sense of cooperation toward achieving a mutual goal of quality science. At no point were regulatory (risk manage-

ment) staff involved; rather, the discussions were scientist to scientist. For both IGB and WIL in-life components of the atrazine amphibian study, OECA concluded that no violations of GLP regulations were observed even though the intent of the inspections was primary to insure that the studies were adhering to protocols designed to address uncertainties identified in previous studies, no violations were noted at the EPL inspection.

A major benefit of the inspections, was that it helped US EPA staff to better understand the logistical constraints of both conducting a study of this magnitude and which also involved non-standardized methods. The inspections helped to foster a level of trust with EPA personnel where the registrant and their contract labs were able to candidly discuss problems/deviations and to scope out ways of addressing them. Although the registrant submitted the study final report well after the agreed due date, OPP was very familiar with the methods and results because of the close communication that occurred during the conduct of the studies. The focus on detail/documentation later allowed the agency to substantiate critical aspects of the study without which OPP may have classified the study as invalid.

CONCLUSIONS

Clearly there is an active debate about the potential effects of atrazine on non-target organisms. However, the skill mix and cooperation between the MA, RA, and the test facilities (TF) allowed OPP to ensure GLP standards were being followed as well as allowing everyone involved to address higher level science issues associated with the study execution. There was an overall emphasis on sound science by everyone involved in the inspection. While the registrant felt that all of the comments from OPP helped to improve the quality of the amphibian studies, the most significant contribution from their perspective was in acquainting the German testing facility, IGB, with the rigor of review applied to FIFRA GLP studies. IGB had not conducted FIFRA GLP studies prior to the atrazine amphibian studies. OPP's inspection in conjunction with our German counterparts was a new experience for IGB. The level of detail and accountability expected from the lab increased significantly as a result of the inspections.

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