SPORTS ANTI-DOPING RESEARCH FUNDING PROPOSAL

Detection of the abuse of exogenous peptide hormones - equine growth hormone and IGF-I analogues.

R. Kazlauskas, G. J. Trout, and C. Howe.

AUSTRALIAN SPORTS DRUG TESTING LABORATORY
AUSTRALIAN GOVERNMENT ANALYTICAL LABORATORIES
1 Suakin St, Pymble, NSW, 2073 Australia

SPORTS ANTI-DOPING RESEARCH FUNDING PROPOSAL

Application Form and Information Requirements

Organisational Details

Legal name of organisation	**Commonwealth of Australia, Department of Industry, Tourism and Resources**
Short name or trading name	**Australian Government Analytical Laboratories**

Type of organisation

	Non-profit organisation		Regional organisation
	For profit organisation		Educational institution
	Registered charity/charitable organisation		Aboriginal or Torres Strait Islander organisation
	Health institution	X	Government
	Community group		Private individual

Postal address

Street name & number/PO box	1 Suakin St.				
Suburb/Town	Pymble				
City	Sydney	State/Territory	NSW	Postcode	2073

Nominated contact for project/program

Title	Dr
First name	Rymantas
Last name	Kazlauskas
Position	Director, Australian Sports Drug Testing Laboratory
Phone	02 94490111
Facsimile	02 94498080
Email address	Ray.kazlauskas@agal.gov.au

Organisation Identification

Australian Business Number (ABN) or Australian Company Number (ACN)

5	1	8	3	5	4	3	0	4	7	9	0	0	2

Is the organisation

GST registered?	Yes	x	No	
Incorporated?	Yes		No	x

If yes, please provide the incorporation number and year of incorporation

Incorporation number											
Date of incorporation											

Purpose/objective/mission statement of organisation (5 lines max)

The Australian Government Analytical Laboratories (AGAL) is the Australian Government's principal agency for the provision of analytical services in chemistry, microbiology, and materials and building science

Information requirements

The following information must be provided with this application

1. Details of any ethical consideration for the project. – **Include a copy of National Health and Medical Research Council approved ethical committee application form, informed consent form, and documentation of the ethical approval process.** ***See attached papers***
2. A detailed budget for the project. This should include: – **A detailed cost item breakdown;** – **Detailes of where the funds will be spent; and** – **Details of any other capital or in-kind support secured for the project.** ***See attached papers***
3. Consultation and/or collaboration arrangements. – **Identify the International Olympic Committee accredited laboratory(ies) that you will communicate or collaborate with to ensure that the new or modified detection protocols and methodologies developed by your research can be implemented by IOC accredited laboratories.** ***Australian Sports Drug Testing Laboratory is the applicant. The methods used are those utilised by IOC accredited laboratories. Regular discussion with IOC laboratories occurs especially at the Cologne Workshop where progress will be reported.***
4. Project summary, suitable for publication (maximum 1000 words) ***See attached papers***
5. Project description. – **This should focus on the expected outcome of the project and the selection criteria (max 5 pages).** ***See attached papers***
6. Project timetable, including proposed milestones. ***See attached papers***
7. Project management plan, including reporting and evaluation plans. ***See attached papers***
8. Other enclosures. **a. Curriculum vitae of principal investigator with 10 relevant, recent publications** **b. Curriculum vitae of main collaborating investigators with 5 relevant, recent publications** **c. List of literature relevant to the project (max 10 publications)** ***See attached papers***

DECLARATION: I declare that, to the best of my knowledge, the information provided in this application is true and complete, and that I have read, understand, and agree to comply with the Guidelines for Applicants.

Signature of CEO or equivalent office holder:

Date:

Project Title

Detection of the abuse of exogenous peptide hormones - equine growth hormone and IGF-I analogues.

1. Ethical Considerations

For these studies no administration of drugs is required. No further ethics approval will be sought or needed.

2. Budget - Request

Project budget	
Expense category	**From ADRP Amount**
Year 2004/2005 **Salary, scientific personnel:** 5% of SPOA plus on costs 10% of SPOC plus on costs 40% of PO1 plus on costs	 **5,500** **8,800** **24,800**
Salary, technical personnel 25% of technician plus on costs	 **15,500**
Consumables: Signal reagents, substrates, capture, sandwich and secondary antibodies for ELISA method validation for detecting eGH and Long R^3 IGF-1.	 **7,000**
Signal reagents, substrates, capture, sandwich and secondary antibodies for ELISA method validation for detecting antibodies to eGH and Long R^3 IGF-1.	**4,000**
ELISA kits for analysing 200 serum samples	**10,000**
Travel to Cologne Workshop to present and discuss results to other WADA laboratories	**3,000**
Other direct costs and overheads (including support infrastructure, equipment maintenance and repairs, services etc)	**49,600**
Total budget	**128,200**

BUDGET JUSTIFICATION

The details of the budget are set out above. The ADRP funds will be used to develop and validate ELISA methods which can be used to detect equine growth hormone and Long R^3 IGF-1 and antibodies to these compounds in serum. This will require four separate assays each of which will require approximately ten ELISA plates. The four methods will be applied to the analysis of some 200 athlete serum samples with a view to determining whether the abuse of these exogenous peptide hormones is occurring in Australia.

4. Project Summary

There is evidence that some athletes are abusing unconventional peptide hormone agents, such as those available for veterinary use, and for use in cell culture. The products include non-human growth hormone, in particular equine growth hormone, and IGF-1 analogues. Whilst there is little or no evidence in the peer reviewed literature that such products are effective performance enhancing agents there is considerable evidence that such products are being abused. Bodybuilders have admitted to their use and seizures of such compounds have been made from athletes. Bodybuilders also claim that they work.

Even if these products are not efficacious there is still a problem with their use by athletes because these hormones have the potential to cause severe harm to those who use them. They are foreign proteins to humans and therefore will induce an immune response. Acute severe immune responses can be life-threatening, and a chronic immune response may interfere with the functioning of the abuser's own growth hormone axis.

The aim of this project is to develop immunochemically based methods to detect equine growth hormone and IGF-1 analogues such as Long R^3 IGF-1 abuse in athletes. Whilst the development of tests for detecting doping with human growth hormone and IGF-1 is complex and ongoing, development of tests for non-endogenous forms of growth hormone and IGF-1 may be relatively simple as the compounds being analysed should not occur naturally.

5. Project Description

Detection of the abuse of exogenous peptide hormones - equine growth hormone and IGF-I analogues.

Australian Sports Drug Testing Laboratory
1 Suakin St., Pymble
NSW, 2073 Australia
Phone 61 2 94490111 Fax 61 2 94498080 email ray.kazlauskas@agal.gov.au or graham.trout@agal.gov.au
Researchers: R. Kazlauskas, G. J. Trout, and C.Howe.

INTRODUCTION

The detection of the abuse of human growth hormone (hGH) and related compounds such as IGF-1 has been the subject of considerable research (Sonksen 2001). The detection of growth hormone is not difficult but to prove that hGH has been administered is very difficult. It has become apparent recently that athletes, particularly from power sports, are abusing unconventional peptide hormone agents, such as those available for veterinary use, and for use in cell culture. This information comes from

- manufacturers, who receive enquiries about their products which are clearly not from legitimate end users.
- publications, especially underground material published in print or online by the bodybuilding community. One story that has been spread via the internet is that the commercial equine growth hormone is actually human growth hormone (Mueller 2001).
- seizure of materials by Customs, drug enforcement and other authorities, that are identified as such agents.

Non-human growth hormones appear to be possible targets for abuse, and this laboratory has identified equine growth hormone (eGH) in vials of material seized by authorities in the past. The ergogenic capacity of these agents is doubtful. Regardless of their intended or other endocrine effects, these hormones have the potential to cause severe harm to humans who use them. They are foreign proteins and therefore will induce an immune response. Acute severe immune responses can be life-threatening, and a chronic immune response may interfere with the functioning of the abuser's own growth hormone axis.
Thus the extent of any abuse of eGH in elite athletes should be established, as much for the health and safety of the athletes as for anti-doping purposes.
IGF-I is consistently identified as a likely drug of abuse in sports. Just as with hGH there is no validated method to detect IGF-I abuse in athletes, because the athlete's endogenous IGF-I confounds the detection of injected IGF-I. There are a variety of IGF-I analogues which are manufactured for experimental use *in vitro.* Some of these have greater IGF-I activity than the native peptide, although some are designed to block IGF-I activity. One that is highly active, and designed to improve the health of mammalian cells in culture, is Long R^3 IGF-I. It

is identified as a desirable agent by bodybuilders in particular (Intensity X 2003). One reason for this may be the paper which states that Long R3-IGF-1 infusion tends to preserve whole-body and muscle protein in beef heifers on a restricted diet.(Hill et al 1999).

Unlike hGH and native human IGF-I, doping with either of these agents should be readily identifiable as neither is present in normal human body fluids.

AIMS

The main aims of the project are to:

- Develop an ELISA based assay for detecting equine growth hormone in serum and possibly urine.
- Develop an ELISA based assay for detecting Long R^3 IGF-I in serum and possibly urine.
- Develop an ELISA based assay for detecting anti-equine growth hormone antibodies in serum.
- Develop an ELISA based assay for detecting anti-Long R^3 IGF-I antibodies in serum.

SIGNIFICANCE

Peptide hormones are banned in sport whether they are of human or non-human origin. Most research conducted so far has concentrated on the detection of human peptide hormones which is very difficult because of the need to distinguish endogenous and exogenous material. Despite it being a much simpler problem to solve, very little has been done on the detection of non-human peptide hormones, probably because it was felt to be less of a problem in sport. However there are now significant indications that the use of non-human products is occurring. Even if there are no proven performance enhancing effects of the use of such materials there are very significant health risks associated with such practices. By developing assays for detecting such materials it will be possible to determine the extent of the problem in the Australian context. These substances can be considered as related to human growth hormone and so are banned under the WADA code.

BACKGROUND

There are two approaches that could be adopted to detect the abuse of non-human growth hormones and IGF-! analogues. The first relies on directly identifying the peptide by immnuoassay whilst the second relies on the detection of an immune response to the peptides in question.

The first approach is the simpler and will be developed initially. Commercially available antibodies to eGH and Long R^3 IGF-I, suitable for ELISA or Western blotting development have been identified and are commercially available. This removes the need to raise antibodies, which is an expensive and time consuming process. Once developed the immunoassay procedures must be validated in the required sample matrices, human serum and possibly urine, and the research effort will initially be directed development and validation. It is much more likely that the method will succeed for serum than for urine.

The rationale behind also using the immune response method is based on the relatively short time that non-human growth hormones and IGF-1 analogues are likely to remain in the body. There is no information available to indicate how long eGH or Long R^3 IGF-I persist in serum after injection in humans, and there are strong ethical indications against trying to obtain that information experimentally. However it is not unreasonable to extrapolate from pharmacokinetic data on human growth hormone or native human IGF-I, where the time frame available to detect the agents is measured in hours not days. As the peptides to be detected are foreign to the human body, it is very likely that they will elicit an immune response in the user, and this response will persist for much longer than the peptides themselves. The method for detecting such antibodies is a related procedure to normal ELISA.

RESEARCH PLAN

The development and validation of the ELISA assays for eGH and Long R^3 IGF-I in serum will take approximately three months and require the use of up to 10 ELISA plates per peptide. A similar amount of time and effort will be required for the antibody assay development. Once the methods are validated it is proposed to use them to measure levels in routine ASDA blood samples.

Project Timeline

Activities	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
2004/2005												
Develop and validate ELISA assay for equine growth hormone.	■	■	■									
Develop and validate ELISA assay for Long R3 IGF-1	■	■	■									
Develop and validate ELISA assay for equine growth hormone antibodies			■	■	■							
Develop and validate ELISA assay for Long R3 IGF-1 antibodies.			■	■	■							
Analyse 200 ASDA blood samples.					■	■	■	■				
Prepare paper for Cologne Workshop								■	■			
Report to ADRP						■						■

Equipment and resources already available for use in this project

Plate washer.

Plate reader and software.

References

Sonksen P. H. (2001). Insulin, growth hormone and sport. Journal of Endocrinology 170, 13-25.

Mueller J (2001). Equigen – the facts. Anabolic Extreme Issue #45 http//www.anabolicextreme.com/anabolic/new_archives/ anex_archive_issue45_EQUIGEN.htm. Accessed 20/1/2004

Intensity X (2003). IGF-1 Long R3. http://www.intense-training.com/forums/t1661.html. Accessed 20/1/2004.

Hill R. A., Hunter R. A., Lindsay D. B., Owens P. C. (1999). Action of long(R3)-insulin-like growth factor-1 on protein metabolism in beef heifers. Domest. Anim. Endocrinol. 16: 219-29.

Curriculum Vitae of principal researchers .

Curriculum Vitae – R. Kazlauskas
Director Australian Sports Drug Testing Laboratory
Australian Government Analytical Laboratory

Family name Kazlauskas
First name Rymantas
Title Dr
Department/school/other
Organisation Australian Government Analytical Laboratories
Postal address 1 Suakin St, Pymble, NSW, 2073, Australia.

QUALIFICATIONS:
B.Sc. 1st Class Honours, University of Sydney. 1968.
Ph.D. University of Sydney, 1972.
Post Doctoral work with Professor A.R. Battersby, University Chemical Laboratory,

EMPLOYMENT HISTORY:

1988-present	Director, Australian Sports Drug Testing Laboratories, Pymble
1986-1988	Research and Development, Australian Government Analytical Laboratories, Pymble.
1982-1986	Senior Research Officer, Department of Pharmacology, University of Sydney.
1981-1982	Visiting Fellow, Research School of Chemistry, A.N.U.
1973-1981	Senior Scientist, Roche Research Institute of Marine Pharmacology

Has been involved in many aspects of research in analytical chemistry since 1971, with a concentration on aspects related to doping in sport since 1988. During this period has published more than 70 papers in refereed journals.

As Director of the only IOC accredited laboratory in the Australasian area since the facility was established at AGAL in 1990 has had responsibility for ensuring that methods were introduced and developed to ensure compliance with the ideals and needs of the IOC anti-doping code. The laboratory was able to obtain IOC accreditation very quickly because of the expertise held within the laboratory. ASDTL are considered the Australian experts, as evidenced by the fact that ASDTL was responsible for analysis of all samples for the 2000 Sydney Olympics.

To maintain this pre-eminent position ASDTL needs to be actively engaged in research into new methodologies both to improve tests for existing drugs and to combat new drugs as they appear. During the past three years has been a contributor to grants totalling in excess of $3 million from the IOC and the Australian Government "Backing Australia's Sporting Ability" initiatives. The World Anti-doping Agency has also agreed to provide funding for a number of projects within ASDTL and for partnerships with other expert groups.

Recent publications

Trout G. J. and Kazlauskas R. (2004) Sports drug testing – an analyst's perspective. Chemical Society Reviews. 33:1-13.

Goebel C., Trout G. J., Kazlauskas R. (2004) Rapid screening method for diuretics in doping control using automated solid phase extraction and liquid chromatography-electrospray mass spectrometry. Analytica Chimica Acta 502:65-74.

Kazlauskas, R. (2002), Analysing the Olympic Games: A case study within the Anti-Doping Programme: An Overview. *Clin. Biochemist Reviews*, 2002; 23(ii): 35

Kazlauskas, R., Howe, C. and Trout, G. (2002), Strategies for rhEPO detection in sport. *Clin. J. Sports Med*. 12(4):2002;229-235.

Gore C. J., Parisotto R., Ashenden M. J., Stray-Gundersen J., Sharpe K., Hopkins W., Emslie K., Howe C., Trout G. J., Kazlauskas R., Hahn A. G. (2003). Second generation blood tests to detect erythropoietin abuse in athletes. Haematologica 88:333-344.

Trout, G., Kazlauskas R. and Westwood, S. (2001). The Role of Reference Standards in the Sydney 2000 Olympic Games Drug Testing Program. *CITAC Newsletter*.

Parisotto, R., Wu, M., Ashinton, M.J., Emslie, K.R., Gore, C.J., Howe, C., Kazlauskas, R., Sharpe, K., Trout, G.J., Xie, M. and Hahn, A.G. (2001). Detection of recombinant human erythopoietin abuse in athletes utilising markers of altered erythropoiesis. *Haematologica* 86: 128-137.

Kazlauskas, R. and Trout, G. (2000). Drugs in sports: analytical trends**.** *Ther. Drug Monit*. 22:103-109.

Corrigan, B. and Kazlauskas, R. (2000). Drug testing at the Sydney Olympics. *Med. J. Austral*. 173:312-313.

Allan, R.D., Dickenson, H.,W., Johnston, G.A., Kazlauskas., R., and Mewett, K.N. (1997). Structural analogues of ZAPA as GABA agonists. *Neurochem. Int*. 30:583-59.

Graham John Trout

Born 6th March 1944.
Tertiary Education:
University of Sydney:

B.Sc. 1965
M.Sc. 1967 "Radiolytic and Non-Radiolytic Decomposition of Methanol"
Ph.D. 1972 "Triboluminescence and Associated Decomposition of Solid Methanol"
Grad. Dip. Ed. 1980

Employment:

1. From 1966 to 1971 I was a full-time Teaching Fellow at the University of Sydney in the Pharmacy Department.
2. From early 1972 to late 1974 I was a Research Scientist in the Central Research Department of Unilever Australia Pty Ltd.
3. From late 1974 to early 1978 I was a Senior Research Chemist and Deputy Research Manager of the Research and Development Division of Australian Newsprint Mills.
4. From 1978 to early 1988 I was a Lecturer of Chemical Instrumentation in the School of Applied Science in the NSW Department of TAFE.
5. From 1988 to 1996 I was the officer in charge of the Gas Chromatography / Mass Spectrometry (GC/MS) Section at the Australian Government Analytical Laboratories (AGAL) at Pymble.. I was also the second in charge of the team developing methods for drug testing in athletes. This work mainly involved the development of GC/MS methods for such testing
6. Since early 1996 I have been the Deputy Director of ASDTL with the primary responsibility of developing new methods including high resolution mass spectrometry (HRMS). This included the management of the $3,000,000 Olympics research program whose aim was to develop new testing techniques for the Sydney 2000 Olympics. Problems under investigation included the detection of low level steroids by instrumental techniques such as HRMS, the detection of administered endogenous steroids by isotope ratio mass spectrometry, and the detection of peptide hormone administration. Our efforts in the peptide hormone field concentrated on the detection of EPO abuse with some effort on measuring growth hormone isoforms in collaboration with the Garvan Institute. In late1999 the International Olympic Committee awarded a competitive research grant of US $1,000,000 to an international consortium led by the ASDTL and the AIS for the development and validation of a test for recombinant EPO. Matching funds were provided by the Australian Government. As a result of these efforts a test was developed and approved for use at the Sydney 2000 Olympic Games. On-going research includes the improved detection of EPO, the detection of growth hormone and the detection of haemoglobin based oxygen carriers

Awards

1990 Department of Administrative Services Award for Excellence (joint)
1991 Australia Day Achievement Medallion
1995 Department of Administrative Services Award for Excellence
2000 Australian Sports Medal
2001 Australia Day Achievement Medallion (joint)

Publications

Co-author of over 25 publications in peer reviewed journals or books, plus numerous conference presentations.

Journal Articles:

Trout G. J. and Kazlauskas R. (2004) Sports drug testing – an analyst's perspective. Chemical Society Reviews. 33:1-13.

Goebel C., Trout G. J., Kazlauskas R. (2004) Rapid screening method for diuretics in doping control using automated solid phase extraction and liquid chromatography-electrospray mass spectrometry. Analytica Chimica Acta 502:65-74.

Alma C., Trout G., Woodland N., Kazlauskas R. (2002). The detection of haemoglobin based oxygen carriers., in Recent Advances in Doping Analysis (10), W. Schanzer et al (ed.), Sport und Buch Strauss, Koln, 2002, 169-178.

Keung K.C., Howe C., Gui L.Y., Trout G., Veldhuis J.D., Ho K.K. (2002). Physiological and pharmacological regulation of 20-kDA qrowth hormone. Am. J. Physiol. Endocrinol. Metab., 283: E838-43.

Trout G.J., Emslie K.R., Howe C., Kazlauskas R., Lasne F. (2002). An overview of testing for EPO at the Sydney 2000 Olympic Games and beyond. in Recent Advances in Doping Analysis (9), W. Schanzer et al (ed.), Sport und Buch Strauss, Koln, 191-200.

Christopher John HOWE

E-mail: c.howe@agal.gov.au
Current Appointment: Manager Peptide Hormones, Australian Sports Drug Testing Laboratory

Qualifications: University of NSW. Bachelor of Science in biochemistry. Graduated 1978.

University of Sydney. Master of Science in Medicine, by research and thesis in the Department of Obstetrics and Gynaecology, Faculty of Medicine. Project: Development of methods for the measurement of the androgens nandrolone and testosterone in human body fluids, and application of the assays to the study of the pharmacokinetics and pharmacodynamics of nandrolone in man. Graduated April, 1996.

Member, Endocrine Society of Australia.

EMPLOYMENT HISTORY

January 1998-Present. Research Officer and now Manager Peptide Hormones, Australian Sports Drug Testing Laboratory.

The initial position was created as a part of the Olympics Research Program, funded by the Australian Commonwealth Government, to improve detection of the misuse of recombinant and naturally derived protein hormones. Two main projects were conducted:

A. Erythropoietin (EPO): A major series of studies was conducted to identify and validate a set of haematological parameters which indicate EPO misuse. In 1999, a pilot study in collaboration with the Department of Physiology at the Australian Institute of Sport identified a combination of factors in whole blood and serum which, suitably weighted and combined, distinguished current and recent use of EPO. In 2000, a study was funded by the Australian Commonwealth Government and the International Olympic Committee to validate the procedure. This study involved a collaboration between ASDTL and the AIS as well as active collaborators in China, France, Norway, Canada and Italy. Blood samples were collected from 1200 elite athletes in 14 countries, as baseline data to test the validity of the test model. The entire study, including data reduction, was conducted between December 1999 and July 2000, for presentation to the Medical Commission of the IOC in the beginning of August. The test procedure was approved for use at the Sydney Olympic Games, together with a procedure for identifying recombinant EPO in urine. I was sent to Paris in July 2000 to transfer this method to Sydney. These procedures are now being established as part of the service assays of the laboratory.

B. Human Growth Hormone (hGH). A smaller study was conducted in collaboration with the Pituitary Research Unit of the Garvan Institute of Medical Research, to investigate the use of molecular weight isoforms of hGH as markers of recombinant human growth hormone abuse. Sera from normal volunteers and patients suffering from a variety of relevant clinical conditions were examined and both the 20 kilodalton and 17 kilodalton forms were confirmed as having potential as markers of hGH abuse.

PREVIOUS APPOINTMENTS

1988-January 1998. Technical Officer and Hospital Scientist (May 1997-January 1998), Andrology Unit, Royal Prince Alfred Hospital, Central Sydney Area Health Service.

Originally employed as scientific support (immunoassay, sperm function analyses and computer support) for the Sydney centre in two multicentre contraception studies conducted by the World Health Organisation Human Reproduction Program. Further developed interests in the physiology and pharmacology of androgens, especially as applied to the development of male contraceptive methods. Completed a Master's degree in the development of methods for the analysis of endogenous and exogenous androgens in human serum, with application to the pharmacology of nandrolone esters in man.

December, 1977-1982. Scientific Officer, Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW 2050.

1982-1988. Senior Scientific Officer, Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW 2050.

SELECTED PUBLICATIONS

Keung K.C., Howe C., Gui L.Y., Trout G., Veldhuis J.D., Ho K.K. (2002). Physiological and pharmacological regulation of 20-kDA qrowth hormone. Am. J. Physiol. Endocrinol. Metab., 283(4), E838-43.

Parisotto, R, Wu, M, Ashenden, MJ, Emslie, KR, Gore, CJ, Howe, C, Kazlauskas, R, Sharpe, K, Trout, GJ, Xie, M & Hahn, A (2001). Detection of recombinant human erythropoietin abuse in athletes utilizing markers of altered erythropoiesis. Haematologica 86:128-137.

Kicman, AT, Coutts, SB, Cowan, DA, Handelsman, D J, Howe, CJ, Burring, S, Wu, FCW (1999) Adrenal and gonadal contributions to urinary excretion and plasma concentration of epitestosterone in men - effect of adrenal stimulation and implications for detection of testosterone abuse. Clinical Endocrinology 50:661-668.

Howe, C, Handelsman, DJ (1997). The use of filter paper for sample collection and transport in steroid pharmacology. Clinical Chemistry 43:1408-1415.

Minto, C, Howe, C, Wishart, S, Conway, AJ, Handelsman, DJ (1997). Pharmacokinetics and pharmacodynamics of nandrolone esters in oil vehicle: Effects of ester, injection site and volume. Journal of Pharmacology and Experimental Therapeutics 281:93-102.