(54) Title: DEACTIVATING AN EXPLOSIVE COMPOSITION USING ENZYMES

(57) Abbrev/Abstract:
A method of deactivating an explosive composition provided in an explosive cartridge, which method comprises exposing the explosive composition to a deactivating agent that renders the explosive composition insensitive to detonation, wherein the deactivating agent is an enzyme used in isolation from any living cell with which it might normally be associated or produced.
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Abstract: A method of deactivating an explosive composition provided in an explosive cartridge, which method comprises exposing the explosive composition to a deactivating agent that renders the explosive composition insensitive to detonation, wherein the deactivating agent is an enzyme used in isolation from any living cell with which it might normally be associated or produced.
DEACTIVATING AN EXPLOSIVE COMPOSITION USING ENZYMES

The present invention relates to a method of deactivating an explosive composition in order to render the composition safe. The present invention also relates to a cartridge that contains an explosive composition and that is adapted to achieve deactivation of the explosive composition in the event that it is not detonated as intended during use.

Explosives are used in a significant number of commercial applications, such as mining, quarrying and seismic exploration. In mining and quarrying a detonator is typically used to initiate a cartriged primer charge that in turn detonates bulk explosive. In seismic exploration a relatively small cartriged explosive charge is initiated using a detonator and the shock waves that are generated are monitored and analysed.

When a charge fails to detonate as intended there are obvious safety and security issues. In that event, it may be possible to recover the charge, although this is not always possible for a variety of reasons. For example, in seismic exploration where charges or trains of charges are positioned and detonated, recovery of undetonated charges can be difficult, especially when the charge(s) is/are positioned in an underground borehole and the borehole has been backfilled, as is common practice. There are therefore instances where undetonated charges remain unrecovered in the field. In such cases, and as a general point, it would therefore be desirable to render safe any undetonated and unrecovered explosive charges. A variety of approaches to address this need already exist.

By way of example, US 3,948,177, describes an explosive cartridge for underwater blasting which is said to be self-disarming in the event of an underwater misfire. The cartridge comprises a closed shell including an internal conduit. Water external to the cartridge is prevented from flowing into the conduit by a watertight seal. The force of a percussion impact initiation can however break the watertight seal thereby allowing water to flow into the conduit and contact with explosive composition contained. In turn, water can dissolve the (nitrocarbonate) explosive possibly also causing it to flow out of the body of the cartridge. The result is desensitisation. Whilst generally useful, a problem with this
approach is that desensitisation is contingent upon some form of specific force associated with a misfire to break the watertight seal. If there is no applied force resulting from a misfire, the cartridge would not be disarmed by the action of water.

5 Other approaches, such as those described in WO 97/19253 and WO 98/55822, rely on the use of micro-organisms to effect bio-remediation of an explosive composition in the event that the composition is not detonated as intended. However, being biological in nature, care needs to be taken to provide the micro-organisms in a form that is active or potential to be active, and care needs to be taken not to destroy the micro-organism thereby rendering them useless. It will also be necessary to supply micro-organisms with suitable nutrients/metabolites in order to sustain them when they are required to be active. Approaches using micro-organisms may also lead to unwanted introduction or leakage of possibly exotic micro-organisms and/or chemicals into the environment. Thus, the use of micro-organisms in this context is not without practical problems.

15 The present invention seeks to provide an alternative approach to rendering safe explosive compositions that does not suffer the disadvantages described above.

Accordingly, in one embodiment, the present invention provides a method of deactivating an explosive composition provided in an explosive cartridge, which method comprises AS contacting the explosive composition with a deactivating agent in a form that renders the explosive composition insensitive to detonation after a predetermined period of time, wherein the deactivating agent is provided in the explosive cartridge and wherein the deactivating agent is an enzyme used in isolation from any living cell with which it might normally be associated or produced. In the present invention, and as will be explained in more detail below, the deactivating agent is an enzyme. The enzyme is used in isolation from any living cell with which it might normally be associated or produced. Unless otherwise stated the term deactivating agent is used to denote such enzymes. Mixtures of enzymes may be used.

30 This definition is intended to embrace naturally occurring or produced enzymes that have been isolated or extracted and synthetic enzymes.

In accordance with the present invention, the action of a deactivating agent on the
explosive composition is responsible for rendering the explosive composition insensitive to detonation, i.e. safe. Herein, unless otherwise evident, when it is indicated that an explosive composition is rendered insensitive to detonation means that the explosive composition has, by action of the deactivating agent, been desensitised at least to the extent that the normal (predetermined) method of initiation of the explosive composition is no longer effective. Thus, for an explosive composition that is known to be detonated using a particular type of initiating device, in accordance with the present invention the explosive charge is rendered insensitive to detonation if it is no longer possible for it to be initiated in that way. The fact that an explosive composition has been rendered insensitive to detonation does not mean that the explosive charge is completely undetonable (although this is of course a possibility). At the very least, the extent of desensitisation effected by the deactivating agent in accordance with the invention results in the explosive composition being insensitive to the initiation means that was otherwise and originally intended to cause detonation of the explosive composition.

In an embodiment of the present invention, as well as deactivating the explosive composition, the enzyme converts the explosive composition (or components thereof) into one or more compounds that are more environmentally acceptable.

When the enzyme is derived from a living cell it may be derived from a microorganism, animal or plant cell. Microorganisms capable of degrading explosive material are known in the art and to the extent that this activity is attributed to enzymes associated with or produced by the microorganism, the microorganism may be a useful source of enzymes for the present invention. Examples of microorganisms that are known to exhibit that ability include *Pseudomonas spp.*, *Escherichia coli*, *Morganella morganii*, *Rhodococcus spp.*, *Comamanos spp.*, and denitrifying bacteria. Suitable *Pseudomonas spp.* microorganisms include microorganisms in the group *aeruginosa, fluorescens, acidovorans, mendocina, cepacia*.

The enzymes used in accordance with this embodiment must be functional under the conditions of intended use.
In the invention the enzyme(s) are used in isolation from the cells that otherwise produce or are associated with the enzyme(s). In this case the enzyme is provided in a substantially purified form or in a cell-free form. The latter may be a cell free extract or the enzyme may be provided as a component of a cell-free composition. The enzyme may be produced and isolated by conventional techniques. Enzymes that are known to be useful in degrading explosive materials are known in the art. As an alternative the enzymes may be synthetic and thus not derived from living cells.

It is also known that certain plants have a phytoremediating/rhizoremediating effect on explosive materials. To the extent that this effect is due to enzymes that are produced by or associated with the living cells of the plant, the plant may be a useful source of enzymes for the present invention.

Laundry and dishwasher detergents may include suitable enzymes for use in the present invention and the detergent may represent a convenient format in which the enzyme is provided into the explosive cartridge. In this embodiment (and generally) it may be appropriate to provide the enzyme with co-factors and the like that are required for the relevant functionality or for potentiating the relevant functionality. Temperature and pH conditions may also need to be taken into account.

In an embodiment of the present invention it may be desirable to employ two different deactivating agents (i.e. with different activities) to effect desensitisation of the explosive composition. In this case one of the desensitising agents acts to degrade the explosive composition to some by-product, with the other deactivating agent acting on the by-product. The latter deactivating agent has the effect of thermodynamically increasing the efficiency of the first deactivating agent due to degradation of the by-product associated with the deactivating activity of the first deactivating agent on the explosive composition. This embodiment may be implemented with more than two deactivating agents, as appropriate. In this embodiment at least one deactivating agent should be as required in accordance with the present invention. The other deactivating agent(s) may be of the same or different type.
Typically, the deactivating agent will itself cause suitable desensitisation of the explosive composition. However, it is also possible that further desensitisation may be achieved through the combined activity of the deactivating agent and another reagent useful in deactivating the explosive composition. The another reagent may be a microorganism, (non-biological) chemical, and/or a plant or plant extract/derivative. In the following, unless context requires otherwise, reference to an enzyme/deactivating agent may be taken as reference to the combined use of the enzyme/deactivating agent and the another reagent.

In this case the relative order of activity of the deactivating agent and the another reagent is not especially critical. For example, the another reagent may degrade the explosive composition into a particular by-product that is then acted upon (degraded) by the deactivating agent, or vice versa. In this case the combined activity of the agent and reagent give a beneficial effect in terms of reaction thermodynamics.

Of course, the deactivating agent and another reagent may have the same general activity with respect to the explosive composition. In this case other reagents may be employed to enhance the thermodynamics of the relevant reaction(s) by consuming reaction(s) by-products.

In one embodiment the another reagent may be a reagent external to the explosive cartridge that will find its way or be introduced into the cartridge during use thereof and that can contribute to desensitisation of the explosive composition. Such reagents may be naturally present in the environment in which the explosive cartridge is to be used. In this embodiment the explosive cartridge will be adapted to allow the relevant reagent to be introduced into or enter the explosive cartridge as required. By way of example, the explosive cartridge may be designed to allow environmental water to enter the body of the cartridge and contact the explosive composition, assuming of course that water has a desensitising effect on the emulsion.

By way of further example, the cartridge may be adapted to allow ingress of naturally-
occurring microorganisms (or other remediating agent(s)), for example water-borne microorganisms, that exist naturally in the environment in which the explosive cartridge is being used and that are capable of remediating the explosive composition contained in the cartridge. The cartridge may be provided with a nutrient source to promote uptake and proliferation of such microorganisms (or agent(s)). In this case water serves as a vehicle that transports the microorganisms into contact with the explosive composition.

Central to the present invention is the nature of the deactivating agent and its use in the context of desensitising an explosive composition provided in an explosive cartridge. In certain embodiments of the invention the explosive cartridge may be of known design. For example, the explosive cartridge may comprise a reservoir (or chamber) in which the deactivating agent is provided and a separate component, typically in the form of a shell (or cartridge,) in which the explosive composition is provided. The reservoir and shell are adapted to be connected to each other. The act of connecting the reservoir to the shell may cause release of the deactivating agent from the reservoir so that the deactivating agent comes into contact with the explosive composition. In another embodiment valve means may be provided between the reservoir and shell, as part of one or both components, to regulate when release of deactivating agent takes place. This type of arrangement is disclosed, for example, in US 5,736,669 and US 5,763,815.

In another embodiment the deactivating agent and explosive composition may be provided adjacent to each other but contact of them is prevented by use of a physical barrier element. Prior to use of the explosive cartridge, that is positioning and priming of the explosive cartridge, the barrier element prevents contact between the deactivation agent and explosive composition. In embodiments of the present invention the barrier element is breached or removed instantaneously when the explosive cartridge is being used in the field. In other embodiments the barrier element remains in place between the deactivating agent and explosive composition when the explosive cartridge is actually positioned and primed but some mechanism for delayed removal of the barrier element is activated. The barrier element may be breached/removed by chemical, mechanical or electrical means. Mechanisms useful in implementation of this embodiment of the invention are known in
the art, for example from US 6,120,627, US 6,660,112, US 6,644,200 and US 7,077,044.

Typically, the external configuration of the explosive cartridge is cylindrical with the deactivating agent and explosive composition occupying respective chambers within the body of the cartridge. In this embodiment the explosive cartridge is invariably sealed so that there is no risk of escape of components, for example, during storage and/or transportation. Sealing may be achieved by conventional techniques depending upon the materials used to form the cartridge. If the cartridge is formed from plastic, the body of the cartridge, including the respective chambers of it, may be formed by injection moulding with the chambers of the cartridge being loaded with the deactivating agent and explosive composition as required, with subsequent sealing (heat sealing, for example) in order to seal the inlets through which these components are supplied into respective chambers in the body of the cartridge.

The cartridge may be made up of independent components that are adapted to be attached to each other as the cartridge is being loaded with respective components and when used in the field. By way of example, the explosive composition may be provided in a chamber that is adapted to be secured to another component comprising a chamber for the deactivating agent. The chamber for the deactivating agent may be of single piece construction, for example formed by injection moulding of a suitable plastics material, and include at least one detonator receiving channel as part of the construction. The chamber for the deactivating agent may be provided as part of a cap well or lid piece for the chamber housing the explosive composition. The individual components may be attached to each other by any suitable mechanism, such as interference (friction) fit, male-female screw threading or clip fitting. In this case the explosive composition may be loaded into the respective chamber and the lid secured to the top of the explosive composition chamber. If the explosive composition is a fluid, the attachment must be such that loss of explosive composition is prevented. However, if the explosive composition is solid in nature, for example when the explosive composition is cast hot and allowed to solidify, the
attachment may be loose fitting, and this may be beneficial in terms of allowing water to
enter the cartridge, as will be explained. The cap well (lid piece) will also generally
include a lid/seal over its open end, and this may also allow water to enter the cartridge.
As a further alternative, rather than relying on separate chambers that are integrally formed
as parts of the cartridge structure, the deactivating agent and/or explosive composition may
be provided in independent containers that are inserted into a rigid cartridge body. In this
case it will be appreciated that the cartridge is made up of at least two independent parts
and that in use the cartridge is assembled from those parts.

The material(s) used to form the cartridge of the invention should not be corroded by or be
reactive towards the deactivating agent and explosive composition to be contained. Thus,
the cartridge will retain its structural integrity.

In one embodiment the barrier element takes the form of an internal wall or internal wall
portion (membrane) separating the chambers containing the deactivating agent and
explosive composition. When this wall or wall portion is breached or removed the
deactivating agent and explosive composition come into direct contact with each other. In
accordance with the invention, this occurs only during use. Thus, in one embodiment the
wall or wall portion may be ruptured by insertion of a detonator into the explosive
cartridge (detonators are invariably used to initiate detonation), or by the act of connecting
one cartridge to another to form a train of cartridges, as is common practice.

With respect to use of a detonator, the cartridge is usually adapted to receive the detonator
in a suitably shaped passage extended axially within the body of the cartridge. The
cartridge may be adapted to receive a single detonator or more than one detonator in
respective, suitably shaped passages. In this regard it should be understood that explosive
cartridges for use in seismic exploration, for example, generally allow inclusion of two
detonators, a primary detonator and a secondary (back-up) detonator in case the primary
detonator does not detonate as intended.

In the embodiment described above the barrier element may extend across this detonator-
receiving passage such that, when the detonator is pushed into position in the cartridge, the wall originally separating the deactivating agent and explosive composition is ruptured thereby allowing these components to come into direct contact with each other. Alternatively, the action of inserting the detonator into the cartridge may cause a separate component to rupture the wall. This component may be a needle-like structure, rigid tube, or similar.

To achieve release of the deactivating agent when cartridges are coupled together in a train, the lower end of the cartridge may include a suitably shaped extension for insertion into the detonator-receiving passage of an adjacent cartridge (of the same design). Insertion of this extension into the detonator-receiving passage has the same effect as inserting a detonator in that the wall/membrane separating the deactivating agent and explosive composition is ruptured. Alternatively, the upper end of the cartridge may include a component that is adapted to be displaced downwardly (or upwardly) when the cartridges are coupled together and that causes the wall membrane to be ruptured. To facilitate attachment explosive cartridges in accordance with the present invention may also include suitable engagement or retaining means. For example, the lower end of the cartridge may include external threads with the upper end including corresponding internal threads thereby allowing adjacent cartridges to be secured to each other. It will be appreciated that the external shape of the lower end of the cartridge is adapted to mate with the upper end of an adjacent cartridge. In the particular embodiment described, the act of engaging and screwing cartridges together may cause rupture of the wall.

In another embodiment the deactivating agent and explosive composition may be provided in separate (sealed) components that are coupled only when the cartridge is to be used. Thus, the deactivating agent may be provided in a sealed cap that is adapted to be attached to a base cartridge portion including the explosive composition. The act of coupling the components together may cause release of the deactivating agent and this may be achieved along the lines already described. In this embodiment the cap containing the deactivating agent may need to be adapted to allow for a detonator to be inserted into the base cartridge portion. Additionally, a train of cartridges would need to be constructed with a cap
containing the deactivating agent provided immediately above each base cartridge portion. Construction of a train of individual explosive charges may be more onerous in this embodiment when compared with embodiments where the deactivating agent and explosive composition are provided in a single (cartridge) structure.

Irrespective of which particular embodiment is employed, the integrity of the barrier element will only be compromised when the detonator is being used in the field. Prior to that point in time the barrier element is intended to remain intact thereby separating the deactivating agent and explosive composition.

In the embodiments described, when breach or removal of the barrier element is instantaneous, the deactivating agent and explosive composition will come into contact with each other straightaway. In this case the deactivating agent will start acting upon the explosive composition immediately. However, in such embodiments for the explosive cartridge to have a period of usefulness, it is important that the deactivating agent does not render the explosive composition insensitive to detonation, or reduce significantly the energy output of the explosive composition, immediately. If it did, the explosive cartridge would be useless, or of little practical use, as soon as the deactivating agent is released from the chamber containing it. It is instead intended that the deactivating agent desensitises the explosive composition after a suitable period of time and by this is meant a period of time after which detonation should otherwise have occurred. Thus, after release of the deactivating agent, the explosive cartridge may need to remain fully detonable (with the energetic output of the explosive composition unaffected or substantially unaffected) for a period of up to a few weeks, preferably for a period of up to a few (e.g. three to six) months. In some instances the explosive cartridge may be required to remain detonable (and useful) for a longer period, for example up to about twelve months. The reaction kinetics associated with the deactivating agent and explosive composition will dictate the rate of which the explosive composition is desensitised. In practice to achieve a useful product the reaction is relatively slow so that the transition between the explosive composition being detonable and non-detonable may be a relatively long one.
In other embodiments of the present invention the barrier element is adapted/designed to be breached or removed only after the explosive cartridge is used. In these embodiments removal/breach of the barrier element is not instantaneous on use of the cartridge, but rather some mechanism is activated that will lead to removal/breach of the barrier element after some predetermined period of time. Taking into account the activity of the deactivating agent this will invariably be a period of time after which desensitisation of the explosive composition is desired due to failure of the explosive cartridge to be detonated, as described above. The mechanism by which the barrier element is removed or breached may be chemical, electrical or mechanical in character.

In another embodiment of the invention the deactivating agent is provided separate to the explosive composition and must be mobilised in order for contact with the explosive composition to take place. In this case the deactivating agent may be provided in any suitable form that is rendered mobile by water that enters or is delivered into the explosive cartridge when used. Thus, the deactivating agent may be provided in dehydrated or dried form such that contact with water results in formation of a solution or suspension of deactivating agent in water. Formation of the solution or suspension renders the deactivating agent mobile. The deactivating agent may also be provided as a gel or viscous liquid that itself is not suitably mobile but that when contacted with water becomes mobile. Herein reference is made to water being used as the vehicle that renders the deactivating agent mobile. Other liquid vehicles may of course be used. Water tends to be convenient as it is generally present in environmental in which the explosive cartridge will be used.

A water-permeable membrane may be used to separate the explosive composition and deactivating agent with the deactivating agent permeating this membrane when mobilised by contact with water. In this regard the water-permeable membrane may be provided with one or more apertures to allow the (mobilised) deactivating agent to come into contact with the explosive composition. The same apertures may also allow water to come into contact with the deactivating agent in order to render it mobile. It may also be possible to implement this embodiment using a water-degradable membrane to separate the explosive
composition and deactivating agent. In this case the deactivating agent may be provided in a water-degradable (or water-soluble) packet or wrapper, formed for example from polyvinyl alcohol. This may simplify design since in this case the encapsulated deactivating agent may simply be positioned on top of or within the bulk of the explosive composition. In these embodiments it is important that the membrane or packet/wrapper that is used is not degraded by the explosive composition.

In this embodiment the explosive cartridge may include one or more inlets (apertures) and/or water-degradable pathways to allow environmental water to flow into the cartridge and directly into contact with the deactivating agent. Additionally, or alternatively, the explosive cartridge may include one or more inlets (apertures) and/or water-degradable pathways to allow environmental water to flow into the cartridge and into contact with the deactivating agent through the explosive composition. In this case the explosive composition may include channels to allow water to migrate to the deactivating agent. The channels may be artificially formed in the explosive composition and/or be naturally occurring given the nature of the explosive composition and the manner in which the explosive composition is loaded into the explosive cartridge. With respect to the latter case, if the explosive composition is delivered into the respective chamber above its melting point and is allowed to solidify subsequently, a network of cracks and fissures may be formed in the solidified form of the explosive composition. Water may migrate through these cracks and fissures. In this embodiment water must obviously be able to enter the explosive composition in the first place, and ways in which this can be achieved are described herein. When a water-permeable or water-degradable membrane is used to separate the explosive composition and deactivating agent, the membrane may define a cavity or cavities that separate(s) the deactivating agent and explosive composition with environmental water entering these cavities when the explosive cartridge is used. As a further variation, water may be supplied into the explosive cartridge immediately prior to use. For example, an explosive cartridge could be suitably submerged in water prior to being positioned in a blasthole or the like, so that the water enters the explosive cartridge as desired. Water may also be delivered into the explosive cartridge through a feed line.
In another related embodiment water or some other suitable solution may be contained in a membrane within the shell of the cartridge and/or the explosive composition, with the membrane releasing the water/solution after some predetermined time.

In a further embodiment the deactivating agent may be provided in contact with the explosive composition, for example the deactivating agent may be distributed through the bulk of the explosive composition. In this embodiment the deactivating agent may be encapsulated or provided in pelletised or granulated form, or the like. This general approach is known in the art in relation to the use of microorganisms as deactivating agent, for example from US 6,334,395 and US 6,668,725.

This embodiment also relies on the need for the deactivating agent to be in contact with water so that it is in a form that will effect desensitisation and/or so that it is in a form suitably mobile to effect desensitisation. As noted above the explosive cartridge may include one or more inlets or water-degradable pathways to allow the introduction of water into the body of the cartridge. Water may be conveyed to, and possibly through the bulk of, the explosive composition by use of a suitably designed water-permeable or water-degradable membrane. The explosive composition may be housed in a chamber (shell) the outer walls of which are formed from a water-permeable cardboard or plastics-based material. When the explosive composition is a solid, such as cast Pentolite, in principle it may be possible to dispose of any outer shell. However, the end of the explosive cartridge may then require a rigid end cap or similar housing to facilitate loading of the cartridge into a blasthole.

In an embodiment of the present invention the explosive composition is deactivated by the combined activity of the deactivating agent as described herein and an additional deactivating agent that enters the explosive cartridge during use thereof. For example, the additional deactivating agent may be at least one microorganism that is present in the environment in which the explosive cartridge is being used and that is capable of acting on the explosive composition in order to convert it into by-products that are at least less detonable, and preferably non-detonable, when compared with the explosive composition...
in its original form in the explosive cartridge.

In an embodiment of the invention the additional deactivating agent acts on the explosive composition to render it more environmentally friendly (non-toxic), as might be useful in practice.

In this embodiment the at least one microorganism may be carried into the explosive cartridge in water present in the surroundings in which the cartridge is positioned (blastholes are typically wet environments). The cartridge may be designed to include apertures or inlets to allow ingress of environmental water, and thus microorganisms, into the body of the cartridge and into contact with the explosive composition. Channels may be provided in and/or around the explosive composition to ensure a suitably high surface area of contact between incoming water/microorganisms and the explosive composition.

In one embodiment the cartridge may include a water-permeable or water-degradable outer shell (membrane) surrounding the explosive composition, possibly with channels or passages extending into the explosive composition. In use water permeates or degrades the shell (and channels/passages when present) thereby allowing the water and microorganisms to come into contact with the explosive composition. At that time the microorganisms begin to act on the explosive composition as intended.

In another related embodiment the cartridge includes a shell and optionally channels/passages formed of a material that will be dissolved by water and/or consumed by microorganisms present in the environment in which the cartridge is used. In this embodiment the microorganisms also have the ability to act on the explosive composition as described above. Desirably the microorganisms have a greater affinity for the material of the shell (and where present channels/passages) so that once the material is breached the microorganism acts on the explosive composition.

In these embodiments the time taken for the microorganism to come into contact with the explosive composition and the rate at which the microorganism acts on the explosive
composition as desired (under prevailing conditions of use) is such that deactivation of the cartridge will not be achieved until a predetermined amount of time has elapsed, prior to which the cartridge would normally have been detonated.

The explosive composition used in the explosive cartridge of the invention is conventional in nature and will be selected based on its ability to be desensitised by the deactivation agent or agents to be used. Examples of explosive materials that may be considered for use in the present invention include trinitrotoluene (TNT), pentaerythritol tetranitrate (PETN), cyclotrimethylene trinitramine (RDX) and cyclotetramethylene tetranitramine (HMX). The explosive composition may be an emulsion explosive, a water-gel explosive or an ANFO or other nitrate-based composition. Other less conventional explosives may also be used such as liquid or gel compositions which are aqueous or non-aqueous and possible containing other explosive components such as perchlorates. Combinations of explosive materials may also be used. For example, the explosive composition may be Pentolite, a mixture of PETN and TNT. The explosive composition may also contain other explosive and/or reactive ingredients, such as RDX and metallic (e.g. aluminium) particles.

In one embodiment of the present invention the explosive composition may be a water-in-oil emulsion. Emulsion explosive compositions typically includes a discontinuous phase comprising a supersaturated aqueous solution of an oxidiser salt (usually ammonium nitrate) dispersed in a continuous oil (fuel) phase. Such emulsions are usually formed by mixing the components in the presence of a suitable emulsifier. In the context of emulsion explosive compositions, the deactivating agent may include any reagent that is capable of breaking or rendering unstable the emulsion, thereby causing it to be insensitive to detonation. Usually, the deactivating agent will have the effect of causing crystallisation of the supersaturated emulsion component (the oxidiser salt in the type of emulsions described). Accordingly, one skilled in the art may select suitable reagents for use as deactivating agent, at least for initial screening, based on a general knowledge of emulsion chemistry and of reagents that are known to cause unwanted crystallisation of (supersaturated) emulsion explosive compositions. Here it is important to note that the present invention seeks to make positive use of reagents that might previously have been
regarded as being detrimental in the context of emulsion explosive compositions. The type of deactivating agent used will usually be selected on the basis of the emulsion explosive composition being used rather than vice versa.

5 The present invention has particular utility in seismic survey applications and in this case the explosive cartridge takes the form of a seismic charge. One skilled in the art will be familiar with the type of explosives in this context.

Embodiments of the present invention are illustrated in the accompanying non-limiting figures, in which:

Figures 1-3 shows a cross-section of explosive cartridges in accordance with the present invention, with Figures 2 and 3 illustrating the same design;

Figures 4 and 5 are perspective views of explosive cartridges in accordance with the present invention;

Figure 6 is a cross-section of an explosives cartridge in accordance with the present invention; and

Figures 7 and 8 are perspective views showing a component of the explosives cartridge depicted in Figure 6.

Thus, Figure 1 shows an explosive cartridge (1) suitable for use in seismic exploration. The explosive composition and deactivating agent remain sealed in their respective chambers (2, 3). Therefore, subject to the stability of the emulsion explosive composition, the cartridge (1) is a storage stable product.

The cartridge also includes a small diameter axial channel (4) extending down within the body of the cartridge (1) from the deactivating agent chamber (3) through the explosive composition. This channel (4) is defined by a wall formed from a polymeric material that
is degradable on contact with the deactivating agent. In the arrangement shown in Figure 1
the channel (4) is empty since the deactivating agent has not been released from the
chamber (3). A seal (not shown in detail) is provided between the deactivating agent
chamber (3) and the channel (4), this seal being designed so that breakage of it will cause
release of deactivating agent from chamber (3) into channel (4) extending through the
explosive composition.

The upper end of the cartridge (1) is adapted to receive a cylindrical detonator (5). When
the cartridge (1) is to be used in the field, this detonator (5) is inserted into a detonator-
receiving channel (6) extending into the body of the cartridge (1). In the embodiment
shown the detonator-receiving channel (6) is provided as an extension of the channel (4).
The action of inserting the detonator into the detonator-receiving channel (6) causes the
seal between the deactivating agent chamber (3) and the channel (4) to be broken thereby
releasing deactivating agent into the channel (4). However, contact between the
deactivating agent and the explosive composition is prevented by the walls of the channel
(4) and the deactivating agent must first penetrate these walls before contacting explosive
composition.

Although not shown, it may be necessary for the design to include some kind of air inlet
(or breather tube) to allow air into the deactivating agent chamber (3) as deactivating agent
flows out. In the absence of an air inlet, flow of deactivating agent may be restricted.
Generally, air will only be allowed into the deactivating agent chamber (3) when the
cartridge is being used, thereby preventing leakage of the deactivating agent.

Surface tension effects of the deactivating agent may also influence design or the
characteristics of the deactivating agent to be used. Although also not shown it may be
useful to allow the deactivating agent once released to come into contact with a wick or
open cell foam that extends down into the channel (4) and that has the effect of
conducting/drawing deactivating agent down into the channel (4).

The walls of the channel (4) are made of a degradable (polymeric) material that may be
hydrolysed by water present in the aqueous deactivating agent. On contact of the
deactivating agent and the walls of the channel (4) the deactivating agent therefore
(slowly) degrades the walls. Whilst the walls remain intact no contact of the deactivating
agent and explosive composition takes place and this delay allows a user of the cartridge
(1) sufficient time to load the cartridge into a blasthole and attempt detonation of the
cartridge (1) as intended. Thus, the functionality of the cartridge (1) remains intact even
though the deactivating agent has been released from the chamber (3) originally containing
it.

After a predetermined period of time (usually selected to be a number of months) the walls
of the channel (4) will have been dissolved/consumed/weakened by the deactivating agent.
The integrity of the walls is therefore lost and the deactivating agent comes into contact
with the explosive composition.

Although not shown in Figure 1 the lower end of the cartridge (1) may also be shaped in
order to be inserted into the detonator-receiving channel of an adjacent cartridge. Thus,
forming like cartridges into a train of cartridges can also result in release of deactivating
agent from the chamber (3) in which it is originally contained. The upper and lower ends
of the cartridge (1) may also contain cooperating features, such as screw threads, to enable
cartridges to be secured together.

In the embodiment described when released the deactivating agent flows into channel (4)
running essentially the entire length of the explosive composition included in the cartridge
(1). This is a preferred arrangement and the volume of the cavity is configured to be such
that in use it will contain sufficient deactivating agent to deactivate the entirety of the
explosive composition (over time). After the wall of the channel (4) has been broken
down by action of the deactivating agent, explosive composition adjacent to the
deactivating agent and thus adjacent to the detonator when positioned in the cartridge will
be first exposed to the deactivating agent. This region of the explosive composition
therefore comes into contact with the highest concentration of deactivating agent thereby
promoting the fastest and most effective deactivation of the explosive composition. Other
arrangements are of course possible.

In an alternative arrangement the deactivating agent flows into an annular cavity provided in the outer periphery of the cartridge body. In this embodiment it will be appreciated that the degradable material is provided on the outer surface of the emulsion preventing contact between the explosive composition and the deactivating agent (when released). When the material is degraded by the deactivating agent, the deactivating agent will contact outer regions of the explosive charge first. However, assuming the cartridge is used with a detonator in a central detonator-receiving passage, this embodiment suffers the potential drawback that explosive composition far removed from the location of the detonator will be deactivated first. There is therefore a greater risk of failure to deactivate the explosive composition if the deactivating agent action does not penetrate radially into the explosive composition (towards the location of the detonator). This embodiment does however have the advantage of a high surface area of contact between the deactivating agent and explosive composition.

As a further alternative, the deactivating agent may flow into a cavity provided over the top of the body of explosive composition provided in the cartridge. However, this embodiment suffers the potential disadvantage of low surface area of contact between the deactivating agent and explosive composition and this can lead to slow and/or incomplete deactivation of the explosive composition. Other alternatives are of course possible within the context of the present invention.

Figures 2 and 3 illustrate another embodiment of the present invention. Figure 2 illustrates an arrangement before release of the deactivating agent and Figure 3 an arrangement when the deactivating agent is released. The Figures show an exploded view of only a portion of the cartridge.

Figures 2 and 3 show an explosive cartridge (1) in the form of an elongate cylinder made of a suitably rigid plastic. The cartridge includes a sealed chamber (2) containing an explosive composition and a further sealed chamber (3) containing a deactivating agent.
During storage and transport of the cartridge (1) the deactivating agent and explosive composition remain sealed in their respective chamber (2,3).

The cartridge (1) also includes a small diameter axial channel (4) extending down within the body of the cartridge (1) from the deactivating agent chamber (3) through the explosive composition. This channel is provided off-centre and is distinct from the channel into which a detonator (5) is provided. The walls of the channel (4) may be formed of a porous material that in use will allow deactivating agent to be communicated to the explosive composition and that has sufficient structural rigidity to define a channel adjacent or through the explosive composition.

At the top (entrance) to the channel (4) there is an arrangement that is designed to cause release of deactivating agent from chamber (3) into the channel (4) when the cartridge (1) is to be used. This arrangement includes an elongate element (7) projecting upwardly from the top of the channel (4). This element (7) may be a tube that is adapted at one end to pierce a correspondingly located (rubber) seal (8) provided on the lower end of the deactivating agent chamber (3). The element (7) communicates at its lower end with a seal (9) provided over the entrance to the channel (4). This seal (9) is made of a material that is degradable on contact with the deactivating agent.

Prior to use the seal (8) is in tact and the seal (8) and element (7) are in close proximity to each other. This arrangement is shown in Figure 2. In use of the cartridge, the deactivating agent chamber (3) is displaced downwards relative to the element (7) and this occurs as a result of engagement of the upper end of the cartridge (1) with an engagement member (10). In the embodiment shown the inner surface of the upper end of the cartridge (1) includes screw threads adapted to engage corresponding screw threads provided on the outer surface of the engagement member (10). The member (10) may be a specially designed cartridge cap or the lower end of another cartridge (1). The action of screwing the member (10) into the top of the cartridge (1) causes the deactivating agent chamber (3) to be displaced downwards. In turn this causes the piercing element (7) to pierce the (rubber) seal (8). Deactivating agent then flows down through the element (7) thereby
coming into contact with the degradable seal (9). This is shown in Figure 3. As already noted, an air inlet or breather tube may be required to ensure flow of the deactivating agent, and surface tension effects may need to be taken into account too. Preferably, the air inlet/breather tube is "activated" only when the member (10) is screwed into the top of the cartridge (1) in order to release the deactivating agent. This prevents leakage of deactivating agent prior to use.

After a predetermined period of time the seal (9) will be dissolved/consumed/weakened by the action of the deactivating agent. The integrity of the seal is lost thereby allowing deactivating agent to drain into the channel (4). The deactivating agent then flows through the porous/permeable walls of the channel and into contact with the explosive composition. The deactivating agent goes on to desensitise the explosive composition thereby rendering it safe.

Figure 4 shows an explosive cartridge (1) useful in implementation of the invention. The cartridge 1 includes explosive composition (11) which typically is in a solid (cast) form, such as Pentolite (typically a PETN/TNT and/or RDX mix). The explosive composition 11 includes the detonator receiving channels (6) that enable the cartridge to be initiated by different sized (diameter) detonators. The cartridge (1) includes an outer shell (12) that is made of a water-permeable or water-degradable material. In the field environmental water will thus permeate or degrade the shell. The shell (12) also defines passages (13) extending into the explosive composition (11). The use of this configuration and type of shell allows environmental water to come into contact with the explosive composition (11), and is thus useful in embodiments of the invention where this is intended/required. The explosive composition (11) includes an enzyme-based deactivating agent. For example, the enzyme may be in contact with and/or distributed throughout the explosive composition (11) in the form of pellets or granules. The pellets/granules may be mixed with the explosives composition (11) before the composition (11) is poured (cast) into the outer shell (12). Additionally or alternatively the enzyme may be provided within the material making up the outer shell (12).
Figure 5 shows another form of an explosive cartridge (1) useful in implementation of the invention. The cartridge 1 includes an explosive composition 11, such as a cast Pentolite explosive, surrounded by a shell (12). An enzyme as deactivating agent may be provided in the explosive composition as described above in relation to Figure 4. The shell 12 is water-permeable or water-degradable, as for the shell discussed in Figure 4. In Figure 2 the shell 12 includes radial members 14 extending into the bulk of the explosive composition. The intention here is that when the cartridge 1 comes into contact with water, water dissolves the shell (12) so that water is conveyed into contact with and through the explosive composition, as required by certain embodiments of the invention described herein. The rate at which the shell (12) dissolves may be controlled by suitable selection of material used to form the shell (12).

The material making up the shell (12), passages 13 and/or radial members 14 may be formed of a material that may be degraded by the action of microorganisms. As the shell (12) is degraded this allows water present in the environment to contact the deactivating agent provided in the explosive composition (11) or shell (12). In turn this renders the deactivating agent suitably mobile and/or active so that the deactivating agent can commence desensitisation of the explosive composition. The microorganisms may also have the effect of acting on the explosive composition to convert it into less detonable or non-detonable by-products and/or by-products that are more environmentally friendly.

Figure 6 shows an explosive cartridge (1) suitable for use in seismic exploration. The cartridge (1) includes an explosive composition (a) and deactivating agent (b) in respective chambers (2,3). The chamber for the explosive composition (a) is in the form of a cylindrical shell comprising wall portions (2') sealed by a base (2''). The explosive composition (a) may be Pentolite, possibly in mixture with RDX and/or aluminium particles. The deactivating agent (b) may be a dishwasher detergent containing enzymes and alkaline salts that are capable of deactivating the explosive composition.

The explosive composition (a) and deactivating agent (b) are separated in their respective chambers by a base plate (14) that is loosely fitted at the lower end of the chamber (3) for
the deactivating agent (b). The plate (14) may be formed of any suitable material such as a polyester or polycarbonate. The plate (14) may be provided with a double-sided adhesive to allow it to be positioned and retained in place – the purpose of the plate is to prevent contact between the deactivating agent (a) and explosive composition (b). That said, depending upon the nature of the deactivating agent and explosive composition it may be possible to dispense with the plate (14) altogether.

The cartridge (1) also includes two detonator receiving channels (5') extending into the explosive composition (a). The cartridge (1) also includes a cap (15) at one end. This cap (15) is sized and shaped to fit, for example by interference fit, into the shell housing the explosive composition.

In practice the cartridge (1) may be provided as separate components that are assembled during loading of respective components and when used in the field. With respect to Figure 6, one component may be integrally formed (by injection moulding of a plastics material) to include and define, the cap (15), the detonator receiving channels (5') and the chamber (3) for the deactivating agent (b) as illustrated in Figures 7 and 8. The base plate (14) and chamber/shell (2) for the explosive composition (a) are separate components. The chamber (2) is made up of a cylindrical tube comprising wall portions (2') and a base (2'') that is attached at a lower end of the tube thereby sealing it.

Figures 7 and 8 illustrate certain components shown in Figure 6. Thus, Figures 7 and 8 show the cap (15), detonating receiving channels (5') and chamber (3) for the deactivating agent formed as a one-piece construction, for example by injection moulding of a suitable plastics material. The chamber (3) for the deactivating agent is sealed by a separate plate (14). The cap (15) comprises a circular wall portion (15a) with a lip (15b) that enables the cap (15) to be secured (by interference fit) into a suitably sized and shaped chamber in which an explosive composition is provided (not shown in Figures 7 and 8). The cap (15) is typically inserted into a tube forming. The wall portions (2') extend above and below the cap (15) once inserted and are adapted to allow attachment of other cartridges or a nose cone, for example by thread fitting. The internal surface of the wall portion (2') may
include a lug or tab to engage the lip (15b) so as to maintain the cap (15) in position. The upper end of the cap (15) is open to allow for insertion of at least one detonator into respective detonator receiving channels (5'). The end of the cap (15c) may be sealed with a suitably sized and shaped lid (not shown) or be formed in an injection moulding process.

5 The cap (15) and/or wall portions (2') may include apertures to allow water to enter the explosive cartridge. As noted the wall portion (2') extending above the position of the cap (15) may receive the lower end of another explosive cartridge to form a train of cartridges. In this regard a surface (15c) of the wall portion (2') may be threaded to mate with corresponding threads provided on the outer surface and at the base of another cartridge. Cartridges may also be coupled by interference fit or by clip fasteners. The cap (15) may include apertures or grooves (not shown) in the side wall thereof extending through the circular wall portion (15a) and lip (15b) through which detonator leads may be passed after a detonator loading.

15 The embodiment illustrated in Figures 6-8 may be implemented as follows. In the orientation shown in Figure 8 the plate (14) is removed and deactivating agent inserted into the chamber (3). The plate (14) is then replaced thereby sealing the chamber (3). The seal is loose in the sense that the chamber (3) is not liquid tight. Still in the orientation shown in Figure 8, a cylindrical tube defining the wall portions (2') of the chamber (2) for the explosive composition (a) is inserted over the cap (15) with the cap (15) being retained in place by interference fit between the wall portion (2') and cap lip (15b).

An explosive composition, such as Pentolite, can then be poured into the open end of the tube, thereby surrounding the chamber (3) and detonator receiving channels (5'). If Pentolite is used it is cast above its melting point and allowed to solidify. Solidification may result in the formation of cracks and fissures extending through the bulk of the explosive composition. This may be desirable as such cracks and fissures allow water to travel through the explosive composition, as may be desired. Once the tube has been suitably filled with explosive composition, and the composition solidified as might be necessary, a base (2'') is attached to the open end of the tube. The base (2'') and wall portions (2') may form a seal by interference fit, male-female screw threading or by clip
fastening.

In use the component so-formed is loaded with one or more detonators with the detonator leads being passed out of the cap (15) or upper part of wall portions (2') as noted. The top end of the cap (15) may itself be sealed using a lid made of water-degradable material (not shown).

In the embodiment described it is intended that the deactivating agent is rendered mobile by water entering the chamber (3) around the edges of the plate (14). The plate may additionally or alternatively include apertures to allow water entry into the chamber (3). Additionally or alternatively, the wall portions of the chamber (3) may also include structures to allow water to enter the chamber (3) (the chamber (3) may itself be made of water-degradable material to facilitate water ingress). Water mobilises the deactivating agent and the mobilised deactivating agent may exit the chamber (3) for contact with explosive composition via the same (or different) route through which water entered the chamber (3).

Water may find its way into the chamber (3) in one or a combination of more than one way, as follows.

Where respective components are joined together, for example the wall portions (2') forming the chamber (2) and the cap (15) or the wall portions (2') and base (2''), the joint may allow water ingress. In this case water would enter the chamber (3) around the plate (14) by migration through the bulk of the explosive composition. The composition must therefore allow water transport by the presence of artificial and/or intrinsic water transport structures.

Additionally or alternatively, water may enter the explosive composition through the walls (2') and/or base (2'') of the chamber (2). One or both of these components may include channels/apertures to allow water entry and/or one or both may be water-permeable or water-degradable. The exact configuration will depend upon the form of, and thus the
containment needs, of the explosive composition.

Additionally or alternatively, water may enter the chamber (3) via the cap (15). Thus, the cap (15) may include channels/apertures extending through the cap (15) and into the chamber (3), for example through an aperture between the inner surface (15c) and the chamber (3). The aperture may itself be sealed by a water-degradable material. Water may enter the cap (15) through loose fitting seals (between the cap (15) and cap lid or between the wall portion (2') and an adjacent cartridge when a train of multiple cartridges is assembled). The apertures/grooves for the detonator leads may also allow water to enter the cap. Apertures/grooves in the upper part of the wall portions (2') may also allow water ingress.

One or more components of the cartridge may be water-degradable, and the degradability may be selective in order to provide enhanced control with respect to intended deactivation of the explosive composition.

Irrespective of the way in which water enters the chamber (3), when the deactivating agent is mobilised it will exit the chamber (3) and contact the explosive composition, thereby commencing deactivation of the explosive composition.

Embodiments of the present invention are illustrated in the following non-limiting example.

**Example 1**

500 ml of water was heated to 45°C in a water bath. Pentolite was added to 200ppm (200mg/L), consisting of 70ppm PETN and 130ppm TNT. Deactivating agent (in the form of commercially available detergent) at the recommended dose rate and at 10 x the recommended dose rate was added as noted in Table 1 below. The resultant solution was then removed from the water bath and allowed to sit at room temperature (21°C) overnight in the dark. Samples were taken and analysed for PETN and TNT. The experiment was
repeated using sodium hydroxide and water as controls. The results are presented in Table 1 below. Table 2 below provides a list of ingredients as declared in relevant Material Safety Data Sheets (MSDSs) for the detergents used in the experiments.

<table>
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<tr>
<th>Reagent</th>
<th>Dose (g/L)</th>
<th>PETN (mg/L)</th>
<th>TNT (mg/L)</th>
</tr>
</thead>
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<tr>
<td>Biozet (standard dose)</td>
<td>0.417</td>
<td>45</td>
<td>83</td>
</tr>
<tr>
<td>Cold Power concentrate (standard dose)</td>
<td>1.67</td>
<td>43</td>
<td>54</td>
</tr>
<tr>
<td>Cold Power rainforest (standard dose)</td>
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<td>41</td>
<td>66</td>
</tr>
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<td>62</td>
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<td>Duo matic (standard dose)</td>
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<td>24</td>
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<td>Dynamo matic (standard dose)</td>
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<td>43</td>
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<td>Fab concentrate (standard dose)</td>
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<td>43</td>
<td>53</td>
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<tr>
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<td>40</td>
<td>45</td>
</tr>
<tr>
<td>Finish Powerball 5 in 1 (standard dose)</td>
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<td>56</td>
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<td>43</td>
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<td>75</td>
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<td>64</td>
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<td></td>
<td></td>
<td>Sodium carbonate</td>
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<td></td>
<td></td>
<td>Sodium tridecyl benzene</td>
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<td></td>
<td></td>
<td>sulphonate (linear)</td>
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<td>Enzymes</td>
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<td>Non-haz ingredients</td>
<td>60 – 100</td>
</tr>
<tr>
<td></td>
<td><strong>Finish Powerball 3 in 1</strong></td>
<td>Sodium triplyphosphate</td>
<td>30 – 60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium carbonate</td>
<td>10 - &lt;30</td>
</tr>
<tr>
<td>Product</td>
<td>Ingredients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Finish Powerball 5 in 1       | Sodium percarbonate 10 - <30  
Sodium silicate <10           
Non-ionic surfactant <10      ।
Proteolytic enzyme <1         ।
Amylase enzyme <1             ।
Non-haz ingredients to 100    |
| Home Brand 3 in 1             | Sodium carbonate 30 – 50  
Sodium percarbonate 15 – 30   ।
Sodium silicate 5 – 15        ।
Sodium carbonate peroxyhydrate <5      ।
Sodium disilicate <5           ।
Fatty alcohol alkoxylate (1)  <5           ।
Fatty alcohol alkoxylate (2)  <5           ।
Proteolytic enzyme <1         ।
Zinc sulphate <1              ।
Amylase enzyme <0.25          ।
Alcohols C12 - C15 ethoxylated propoxylated  <0.1 |
| Morning Fresh 5 in 1          | No information as yet                                                        |
| Napisan Plus                  | Sodium carbonate 30 – 60  
Sodium percarbonate 10 - <30   ।
Sodium silicate <10           ।
Anionic surfactant <10         ।
Proteolytic enzymes <10       ।
Non-haz ingredients to 100    |
| Omo matic                     | Alkali salts 10 – 30  
Enzymes 0 – 10                ।
Non-haz ingredients to 100    |
| Radiant Micro concentrate    | No information                                                              |
| Radiant Power concentrate    | No information                                                              |
| Woolworths dishwasher tablets 5 in 1 | No information confirmed as yet, however, same MSDS supplied as for Homebrand |
| Woolworths laundry powder Advanced | No information confirmed as yet, however, same MSDS supplied as for Homebrand |
| Woolworths laundry powder Front Loader | No information confirmed as yet, however, same MSDS supplied as for Homebrand |
| Spree concentrate Apple Fresh | Sodium sulphate  
Sodium carbonate               |
<table>
<thead>
<tr>
<th></th>
<th>Pentasodium triphosphate</th>
<th>Sodium silicate</th>
<th>Tetrasodium pyrophosphate</th>
<th>Sodium hydroxide</th>
<th>Non-haz ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Squeek 4 in 1</strong></td>
<td>No information as yet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Surf Tropical</strong></td>
<td>Alkali salts</td>
<td>Non-haz ingredients</td>
<td>10 – 30 to 100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1 demonstrates that commercially available washing detergents possess a TNT converting ability. The nature of this conversion may involve, amongst other reactions, chemical reduction of one, or more, of the nitrate groups on the TNT molecule to amines, a well established reaction observed in nature. One result of this conversion of TNT is a loss of part, or all, of the Pentolites explosive potential and a rendering of the device less prone to initiation.

This conversion of TNT may also enhance the biodegradation of the device, due to removal of TNT, a chemical known in the art, to be toxic to living organisms, including soil borne microbes.

This conversion of TNT as demonstrated in Table 1, whilst occurring in the presence of strong base, as shown with the sodium hydroxide control value, is enhanced by the presence of enzymes in the commercial detergent preparations. It is accepted that enzymes accelerate chemical reactions including various conversions of PETN and TNT. It is also known that enzymes possess the potential to interact with chemicals other than their intended, or preferred substrate. One example of this is the action of PETN reductase on TNT, two functionally related, but structurally unrelated compounds.

Thus the ability of non-TNT or PETN specific enzymes contained in detergent formulations including, but not limited to, proteases, amylases, lacasses and other unspecified enzymes, to convert TNT can be explained.

**Example 2**

**Enzymatic degradation of Oxalate**
It is established that oxalate is the major alkaline degradation product of TNT. It is thus thermodynamically favourable to remove this end product in order to increase the efficiency of alkaline degradation of TNT, or other aromatics.

Thirty commercially available enzymes were selected for screening to determine their level of oxalate degradation. Apart from commercial availability, other criteria relevant to selection of enzymes for consideration were cost per application and lack of co-factor requirement. Oxalate degrading enzymes previously reported in the scientific literature did not meet these criteria.

Two enzymes, Papain (Enzyme Solutions, Australia) and Bromelain (Enzyme Solutions, Australia) reproducibly demonstrated oxalate-degrading activity (see the following table).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Oxalate (mM)</th>
<th>Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papain</td>
<td>0.030</td>
<td>97%</td>
</tr>
<tr>
<td>Bromelain</td>
<td>0.732</td>
<td>27%</td>
</tr>
<tr>
<td>Control</td>
<td>0.997</td>
<td>0%</td>
</tr>
</tbody>
</table>

Experimental

One ml reactions were performed to demonstrate oxalate degradation in 10 mM potassium phosphate (pH 7.4) buffer containing 1 mM sodium oxalate (Sigma Aldrich, Australia) in water. Reactions commenced with the addition of 10 mg enzyme and were incubated at room temperature on a rotating mixer for 16 hours. In parallel, 1 mM sodium oxalate was incubated in the absence of enzyme as a control. Following incubation, reactions were heated to 85°C for 30 min. Samples were taken for oxalate concentration determination after sample centrifugation at 16100 x g for 5 min.

Oxalate determinations were performed immediately using an Oxalate detection kit (Trinity, Ireland). During the reaction, oxalate was oxidised to carbon dioxide and
hydrogen peroxide by oxalate oxidase. The hydrogen peroxide was measured by its reaction with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3-(dimethylamino) benzoic acid (DMAB) in the presence of peroxidase to yield an indamine dye, which has an absorbance maximum at 590 nm. The intensity of the color produced is directly proportional to the concentration of oxalate in the sample.

To determine oxalate concentration, 10 µL of sample was mixed with 200 µL of oxalate reagent A and then with 20 µL oxalate reagent B. Samples were measured in duplicate. 10 mM potassium phosphate buffer was used as a blank control. Standard curves were generated using 0.1, 0.2, 0.5, 1 and 2 mM sodium oxalate (Sigma Aldrich, Australia). After incubation for 5 min at room temperature, absorbance was measured at 595 nm using a FLUOstar OPTIMA 96 well spectrophotometer (BMG Labtech, Australia).

Example 3

Enzymatic degradation of RDX

The cyanuric acid hydrolase enzyme (AtzD) participates in the degradation and mineralisation of atrazine, a triazine compound commonly found in the environment due to its large-scale use as a herbicide. The pathway of atrazine degradation is encoded by a series of enzymes notated as AtzA, AtzB, AtzC, AtzD, AtzE, AtzF in order of sequential action on atrazine metabolites. (Martinez, B., J. Tomkins, L. P. Wackett, R. Wing, and M. J. Sadowsky. 2001. Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from Pseudomonas sp. strain ADP. J. Bacteriol. 183:5684–5697). Of note is that the enzymes following AtzD serve to degrade Cyanuric acid to carbon dioxide and ammonia, thus AtzD is a triazine ring breaking enzyme.

The following schematic demonstrates the rationale for use of AtzD in RDX degradation due to the similarities in their chemical structures.
Experimental

The DNA encoding AtzD was cloned into the plasmid vector pET-14b (Novagen, Australia) and produced by over-expression in *E.coli* with IPTG induction by standard methods (Sambrook J & Russell D. 2000. Molecular Cloning: A Laboratory Manual [Third Edition]). The AtzD from *Pseudomonas* sp. strain ADP (GENBANK accession no. AAK50331) was used in the present example, however, as it is isofunctional with TrzD (GENBANK accession no. AAC61577) and selected sequence homologues these can be interchanged.

RDX stock solutions were prepared in acetone at 40 mg/ml. Enzyme reactions were performed in Reconstituted Natural Water (RNW), which contains 1 mM KHCO3, 0.5 mM CaCl2, 0.206 mM MgSO4, 8.95 μM FeSO4, and 0.25 mM HCl in MilliQ water in order to mimic environmental conditions.

RDX was added to a final concentration of 20 ppm into 5 ml RNW. Reactions were started with the addition of 100 ppm AtzD enzyme lysate freeze-dried powder (38.1 IU/g). Samples were incubated at room temperature for 48 h and then stopped by the addition of 5mL acetonitrile. Analysis was then performed by HPLC.
Degradation of RDX by AtzD

<table>
<thead>
<tr>
<th>Sample</th>
<th>RDX (ppm)</th>
<th>RDX degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100ppm AtzD</td>
<td>4.6</td>
<td>51.6%</td>
</tr>
<tr>
<td>Control</td>
<td>9.5</td>
<td>0%</td>
</tr>
</tbody>
</table>

Example 4

5 Degradation of PETN with Nitrate Ester Reductase

Purification and Identification of nitrate ester reductase

A bacterial strain, identified by 16S rDNA sequencing as belonging to the species *Agrobacterium tumefaciens* and denoted PD31 (PETN Degrading) was isolated from using methods known in the art. The 16S rDNA sequence was amplified using the DNA primer pair 616V (5’- AGAGTTTGATYMTGGCTC) and 1492R (5’- GGYTACCTTTGACGACCTT). The complete DNA sequence encompassed by these primers is shown below. This DNA sequence was used to interrogate the DNA sequence databases and found to be identical to that of *Agrobacterium tumefaciens* 16S rDNA (http://blast.ncbi.nlm.nih.gov/Blast.cgi) This microbe was isolated via its ability to grow in a culture medium limited to PETN as the only nitrogen source.

**PD31 16s rDNA sequence**

```
1    gagggggggg gcttacagt cagtcgaacg ccccgcaagg ggaqtgqcaq
gaggtgagt aacgcgtgagg acatacctt ttctcgccga ataqctccgg
51   gaaactggaa ttaataccgc atacgcctta cgggggaaag atttatcggg
101  gaaggatggt cccgcgttgg attagctagt tggtggtgta aaggtctacc
151  aaggccagca tccatacgtg ttcgaggaag atgatcagcc aatattggac
201  tgagacacgg ccctaatcctt tacggagagg acgcgtgggg aatatagggc
251  aatgggcgcac agctgtatccg agccatgcgg cggtggtgtg gaaacgttta
301  gggttgtaaa gctctttcag cggagaagaat aatgaccttg cccggagaag
351  aagcccccgga taacctgtcg ccagcagcgg cggtaataag aagggggcga
401  cgctgttccgg gaatattcgg gcgtaaagcg caacgtgggg gatataaag
451  tcaggggtqg aatccccagag ctcacgtctg gaactcgctt tgatactttg
501  tctactggtga ataaaccgag ctcacgtctg gaactcgctt tgatactttg
551  tatcttggtg atggaagagg taatggaat tccagqgtga gatgtqaatg
601  tctgatgatc tggaggacac acctggtggc aaggggcttt actgtccatt
651  tctgatcgtg gaggtgcaga agcggtgggg gcaaaacagaa ttgatctacc
701  tggtgtctca cgcctgaaca gataaatgtt acgcgcttgg caqtaactcg
751  ttgctgctggc cagctaacgc attaaaaccatt ccgcctgggg agtaggcgtcg
801  caagatattaa aacctaaaggta atggacggag ggccgcaacc gcqgtgggacg
851  atggtgttta atgctgaaga cgcggcagaa ccttacgcc actgtcctcttg
901  cggggtatgg gcattggaga gcgtgctcttg cagttaggt gcgcggcgaga
```
Agrobacterium tumefaciens PD31 16SrDNA sequence amplified by 616V/1492R primer pair

This microbe (PD31) was not observed to have any specific growth requirements and was routinely cultured in non-defined media, such as PCA (0.5% peptone, 0.25% yeast extract, 0.1% glucose, with addition of 2% agar as required). Growth of this microbe was observed at all temperatures tested (20-37°C). The PETN degrading capacity of PD31 was not inducible, in contrast to other nitrate ester degrading strains. PD31 Nitrate Ester Reductase (NER) activity was nicotinamide co-factor dependent, however, in contrast to many bacterial NERs, PD31 preferred NADH to NADPH (Table 1).

Enzymatic NER activity was determined by monitoring nitrite production in 50 mM potassium phosphate buffer (pH 7.2), containing 0.2 mM PETN, and 0.2 mM NADH in a final volume of 0.9 ml; 100 μl of enzyme solution was added to begin the reaction. Assays were incubated at room temperature for 5 minutes and then stopped by addition of phenazine methosulfate (0.2 mM, final concentration) and ferricyanide (0.5 mM, final concentration). Nitrite concentration was determined using the Aquanal-plus nitrite kit (Sigma, Cat. No. 37410) as per manufacturers instructions. One unit of activity was defined as the amount of enzyme required to release 1 μmol of nitrite per min from PETN under standard assay conditions.
GTN/PETN degrading activity of cell extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>GTN (IU/mg protein)</th>
<th>PETN (IU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Cofactor</td>
<td>NADH</td>
</tr>
<tr>
<td><em>Agrobacterium</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>tumefaciens</em></td>
<td>1.65x10⁻³</td>
<td>4.15x10⁻²</td>
</tr>
<tr>
<td>PD31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PD31 cells grown for 40 hours in 2xYT medium (tryptone 16 g/L, yeast extract 10g/L, NaCl 5g/L, glucose 2g/L) to an optical density at 600 nm of 5.44 were harvested by centrifugation (5000xg, 20 minutes) and resuspended in 125 mM Sodium Phosphate (pH 7.2) buffer containing the protease inhibitor mix (Complete – Roche, Australia). Samples were disrupted at 700 bar for 2.5 min using an Emulsiflex-C50 High Pressure Homogenizer (Avestin, Inc. Ottawa, Canada). The lysate sample was then clarified by centrifugation (10,000xg, 30 min) and passed through a 0.2micron filter. Saturated ammonium sulphate solution was added to the supernatant to a final level of 40% saturation. The sample was then incubated at 4°C for 1.5 hours, followed by centrifugation at 10,000 x g for 10 min at 4°C to sediment non-NER proteins. The ammonium sulphate concentration was then adjusted to 60% saturation with further addition of saturated solution and incubated for a further 1 hour at 4°C. This sample was then further centrifuged (10,000xg, 30 minutes) and the pellet resuspended into 50 mM Sodium Phosphate (pH 7.0). At this stage the sample contained >50% PD31 NER protein and may be used in the degradation of PETN once appropriately stabilised.

All NER activity appeared to localise to a single protein.

To determine the identity of the NER protein in order to isolate its gene for use in recombinant production of this enzyme, the lysate sample was further fractionated by column chromatography. The sample was applied to a column packed with Sephadex G-100SF size exclusion resin (Amersham Pharmacia, Sweden) by techniques known in the art. The applied proteins were eluted with 50 mM Sodium Phosphate (pH 7.0) buffer in
order to maximise stability of the NER. Protein fractions were collected from the column and
analysed for NER activity. One fraction, with the majority of the NER activity was further
resolved by 12% SDS-PAGE, using standard techniques in the art, and found to contain a
protein of approx. 40 kDa. This protein was excised from the SDS-PAGE gel and it’s N-
terminal sequence elucidated by standard techniques (Australian Proteome Analysis
Facility, Macquarie University, Australia). The N-terminal sequence determined from this
sample was: XX LFEPAAQAG. This sequence was found to be homologous with two genes
of similar reported function, the Glycerol TriNitrate reductase of *Agrobacterium
radiobacter* (EMBL accession: Y13942) and an oxidoreductase of *Agrobacterium
tumefaciens* C58 (GENBANK: NP_355149).

**Example 5**

**Degradation of PETN with Nitrate Ester Reductase**

**Identification, characterisation and expression of *Agrobacterium tumefaciens* PD31
nitrate ester reductase**

The N-terminal protein sequence of the *Agrobacterium tumefaciens* PD31 NER enzyme
(XXLFEPAAQAG) was analysed in conjunction to the DNA encoding two homologous
proteins (Glycerol TriNitrate reductase of *Agrobacterium tumefaciens* [EMBL accession:
Y13942.1] and an oxidoreductase of *Agrobacterium tumefaciens* C58 [GENBANK:
NP_355149]) to design nucleotide primers for the amplification of the encoding gene.
These primers are shown below.

**Nucleotide primers for amplification of the complete PD31 NER gene**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'→3')*</th>
<th>Homology</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRDFNC</td>
<td>GCACCATGGCCAGTCTTTTCGAACC</td>
<td>5' of <em>ner</em></td>
</tr>
<tr>
<td>GRDFND</td>
<td>GCACATAGCCAGTCTTTTCGAACC</td>
<td>5' of <em>ner</em></td>
</tr>
<tr>
<td>GRDRBA</td>
<td>ATCGGATCCCTATTTGGGCGAGGGCCGGATAGTC</td>
<td>3' of <em>ner</em></td>
</tr>
<tr>
<td>GRD2RBA</td>
<td>ACTGGATCCTCAGCCGAGTGCCGGATAGTC</td>
<td>3' of <em>ner</em></td>
</tr>
</tbody>
</table>
GRDR3 | GGTCGCTGCTTTGCCTGCA | 3’ flanking region
---|---|---
GRDF | CCAATCTCTGAGCCTCCCAAG | 5’ flanking region

* DNA restriction enzyme sites are shown underlined and are not homologous to the PD31 sequence, but are included to facilitate cloning of the gene.

The nucleotide primers shown above were used to amplify (by polymerase chain reaction) relevant sections of *Agrobacterium tumefaciens* PD31 DNA molecule. Methods known in the art were also used to purify and sequence this amplified DNA, the commensurate DNA sequence is shown below.

| 1 | atgacaacagtcttttcgaaaa ggcacaggcc ggcgatactg cacatcgcgaac |
|---|---|---|
| 10 | ccggtatcgctatggctcccc cgtcgccgcaaa caagccgctgc gagcactggg gcgtacatcc |
| 15 | ttaaatcctggtcgcc agctgtgca gtcgcggtgcttc gagaacatgg cagcgtcatc |
| 20 | acggcagcagggagcgtctt gcgcgtccgg ggtgtcggactt gcgccttcgg |
| 25 | gatggcgggaaa aagagatcgcgc ccccgtggaactt ggctccggtttaac |
| 30 | tgaaggtgca acgcgcggct cagcatcggg gacaacatggg gacccgttcttc |

Coding DNA sequence of the *Agrobacterium tumefaciens* PD31 enzyme. Flanking regions are omitted.

This DNA sequence once translated yields the protein sequence shown below.
Protein sequence translated from the **ner** sequence. The amino-acids homologous to the experimentally determined N-terminus are shown in bold and underline.

This sequence is unique in the protein sequence databases searched (Non-redundant at http://blast.ncbi.nlm.nih.gov) and as anticipated from the N-terminal sequence, shares homology with the GTN reductase of *Agrobacterium tumefaciens* and an oxidoreductase of *Agrobacterium tumefaciens* C58.

In order to demonstrate the functionality of PD31 NER, nucleotide primers shown above were used to amplify and clone **ner** into the protein expression plasmid pET-14b (Novagen, Australia) by methods known in the art. Four separate recombinant proteins were expressed, corresponding to different terminal sequences. Possible terminal sequence parameters were: presence or absence of a histidine ‘tag’, or a polymorphism at the end of the enzyme inserted by the reverse primers shown in the table above. The NER activity data is shown below and demonstrates the function of this protein.

NER activities of various forms of recombinant *Agrobacterium tumefaciens* PD31 NER enzyme

<table>
<thead>
<tr>
<th>5’ (oligonucleotide)</th>
<th>3’</th>
<th>Optimal Induction (mM)</th>
<th>IPTG</th>
<th>Activity (IU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>GRDRBA</td>
<td>0.05</td>
<td>0.821</td>
<td></td>
</tr>
<tr>
<td>His Tag</td>
<td>GRDRBA</td>
<td>0.05</td>
<td>0.550</td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>GRD2RBA</td>
<td>0.05</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>His Tag</td>
<td>GRD2RBA</td>
<td>0.025</td>
<td>0.952</td>
<td></td>
</tr>
</tbody>
</table>
Enzymatic NER activity was determined by monitoring nitrite production in 50 mM potassium phosphate buffer (pH 7.2), containing 0.2 mM PETN, and 0.2 mM NADH in a final volume of 0.9 ml; 100 µl of enzyme solution was added to begin the reaction. Assays were incubated at room temperature for 5 minutes and then stopped by addition of phenazine methosulfate (0.2 mM, final concentration) and ferricyanide (0.5 mM, final concentration). Nitrite concentration was determined using the Aquanal-plus nitrite kit (Sigma, Cat. No. 37410) as per manufacturers instructions. One unit of activity was defined as the amount of enzyme required to release 1 µmol of nitrite per min from PETN under standard assay conditions.

The data shown in above clearly demonstrate that the gene isolated does indeed encode a NER and further, can be improved by varying its sequence and induction conditions.

The enzyme with the highest level of activity (Native 3’ and GRD2RBA 5’) has been used in all further studies where NER is used.

The recombinant NER enzyme was found to be similar to the wild-type enzyme in terms of thermal inactivation and retained approximately 50% of its activity when incubated at 85°C in solution. This thermal stability was improved when freeze-dried NER was used.

The pH stability of NER is of relevance to its application and was assessed using 50 mM buffers at various pH values (Buffers used: Sodium acetate buffer for pH 4.5-5.5; Potassium phosphate buffer for pH 6-7.6; Tris buffer for pH 8-9; Triethanolamine buffer for pH 8.5; and Sodium carbonate buffer for pH 10-11).

As shown in below, the *Agrobacterium tumefaciens* PD31 NER enzyme has a very wide pH optimum which is a distinct advantage to the application of this in the degradation of Pentolite.
PETN degrading activity of recombinant *A. tumefaciens* PD31 nitrate ester reductase at different pH.

5 Example 6
Degradation of Pentolite with Nitrate Ester Reductase

Recycling of NADH

10 The *Agrobacterium tumefaciens* PD31 NER, like all related enzymes, is a nicotinamide (NADH) cofactor-dependent oxidoreductase. It contains a flavin mononucleotide non-covalently bound to the mature enzyme and catalyses the reductive cleavage of the nitrate ester group to yield an alcohol and liberate nitrite.

15 Due to this reaction mechanism, one mole of NADH is required to cleave each accessible nitrate group. The high cost of NADH and other reported co-factors renders the application of these enzymes non-commercially viable in all but specialty applications.

In order to reduce the cost per application of this enzyme, recombinant production of NER in *E.coli* was accomplished, however, viable co-factors could not be identified. An alternative to this was the re-cycling of the native NADH co-factor, or indeed use of the cheaper and more stable NAD+ that may be converted to NADH *in situ*. 
A variety of enzymes able to reduce NAD+ are reported in the scientific literature, with a majority applicable in some form to the degradation of nitrate esters. An assessment was performed on a number of these enzymes and the *Pseudomonas fluorescens* D-galactose dehydrogenase (GDH) was considered the best candidate on the basis of its relatively innocuous substrate. D-galactose is compatible with most explosives and has the added benefit of providing an excellent carbon source for continued biodegradation by native soil bacteria.

**Experimental**

NER was produced as per previously described in this document.

*Pseudomonas fluorescens* strain 283/2 was kindly provided by RMIT University (Australia). For the non-recombinant production of GDH, one colony from an overnight culture was inoculated in 10 ml minimalM9 medium with 5 g/l D-galactose and 1 g/l ammonium sulphate. After 48 h incubation at 30°C, 200 rpm, 10 ml culture was harvested and pellet resuspended into 1.5 ml 50 mM Tris (pH 7.2) buffer with 100 mM NaCl. The resulting cell suspension was sonicated as is known in the art and D-galactose dehydrogenase activity of the supernatant was determined.

D-galactose dehydrogenase activity was assayed by monitoring the formation of NADH at 340 nm in a 1-ml reaction mixture consisting of 50 mM Tris buffer (pH 8.0), 2 mM NAD, and 0.3% (w/v) D-galactose. One unit of activity was defined as the amount of enzyme required to convert 1.0 μmol D-galactose to D-galactonate per minute at pH 8.0 at room temperature.

In order to generate the efficient recycling of NADH the following procedure was employed. Reactions were set up with 0.2 mM PETN or 100 ppm Pentolite, 23 IU/L NER, 26.2 IU/L GDH, 11.1 mM D-galactose and NAD* at concentrations of 0.02 or 0.002 mM in 50 mM Tris pH 8.0 buffer. Controls omitted the addition of NER. Reactions were
incubated at room temperature for 16 h. The samples were then diluted with 3 parts acetonitrile and PETN/TNT were estimated by HPLC-UV analysis.

PETN and Pentolite degradation by purified PD31 recombinant NER using a co-factor recycling system

<table>
<thead>
<tr>
<th>Sample</th>
<th>NAD+ (mM)</th>
<th>PETN (mM)</th>
<th>PETN degradation</th>
<th>TNT (mM)</th>
<th>TNT degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PETN control</td>
<td>0.02</td>
<td>0.192</td>
<td>0%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>PETN</td>
<td>0.02</td>
<td>0.081</td>
<td>57.8%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>PETN</td>
<td>0.002</td>
<td>0.090</td>
<td>53.1%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Pentolite control</td>
<td>0.02</td>
<td>0.119</td>
<td>0%</td>
<td>0.220</td>
<td>0%</td>
</tr>
<tr>
<td>Pentolite</td>
<td>0.02</td>
<td>0.005</td>
<td>95.8%</td>
<td>0.026</td>
<td>87.7%</td>
</tr>
<tr>
<td>Pentolite</td>
<td>0.002</td>
<td>0.037</td>
<td>68.9%</td>
<td>0.211</td>
<td>4.3%</td>
</tr>
</tbody>
</table>

The table clearly demonstrates an example of the use of commercially feasible quantities of co-factor to drive degradation of Pentolite. It is noteworthy that the reaction proceeds to the point that TNT, a non-ideal substrate for NER is degraded.

Example 7

Degradation of Pentolite by Xanthine Oxidase

Xanthine oxidase (XO) and its alternate form xanthine dehydrogenase diverge in a number of ways including the formers preference for oxygen and the latters for NAD+ as co-factors. Xanthine oxidase (XO) catalyses the oxidation of xanthine to uric acid, thus liberating two electrons and converting molecular oxygen and water to hydrogen peroxide. An alternative process can begin at hypoxanthine which is also converted to uric acid, in a two-step process generating twice the electrons and hydrogen peroxide per mole (see the schematic below).
Of particular interest in this example is XO’s co-factor promiscuity, allowing it to utilise a diverse range of electron acceptors, including nitrates. This property has been harnessed in this example of Pentolite degradation.

This example is significant as it demonstrates an enhanced degradation of PETN when presented as formulated Pentolite. The exact mechanism for this is unclear, however, is most likely to occur due to reaction of free nitrates, TNT, PETN, inorganic or organic constituents of the Pentolite formulation. The interaction of these with XO or its reaction products through reactive oxygen species, or other intermediates may initiate a cascade whereby breakdown products are in turn reactive. This process may be synergistic or truly catalytic under appropriate conditions.

This example further demonstrates that both Xanthine and Hypoxanthine oxidation can lead to efficient degradation of Pentolite. Whilst hypoxanthine can be postulated to supply twice the degrading capacity, it is quite soluble and may dissolve faster than the target compounds, in this case PETN and TNT, thus limiting its utility in high water flow situations. Xanthine, which is significantly less soluble, would thus provide a rate of release more analogous to PETN and thus prove more effective in target degradation.

The relative quantities of Xanthine and Hypoxanthine will thus need to be adapted on the basis of a number of factors, including target solubility and environmental conditions. This
example demonstrates one of these possibilities.

**Experimental**

5 Bovine milk xanthine oxidase (Cat. No. X4875) was used in the examples below and was obtained from Sigma-Aldrich (Australia). Degradation assays were performed in 50 mM potassium phosphate buffer (pH 7.2) with either 0.1 mM PETN or 100 ppm Pentolite as target substrate. Assays were performed in a volume of 3 ml and incubated for overnight at room temperature in the dark. Samples were then prepared for analysis by HPLC-UV by addition of 9 ml acetonitrile.

The following table demonstrates the degradation of PETN when 10 mM xanthine is used as the oxidase substrate.

<table>
<thead>
<tr>
<th>Sample</th>
<th>XO activity (IU/L)</th>
<th>PETN (mg/L)</th>
<th>PETN degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthine Oxidase</td>
<td>500</td>
<td>2.8</td>
<td>66.3%</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>8.3</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

The following table compares the degradation of PETN as sole electron acceptor to the degradation of PETN as formulated Pentolite. Hypoxanthine (10 mM) was used as the oxidase substrate.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PETN presentation</th>
<th>PETN (mg/L)</th>
<th>PETN degradation</th>
<th>TNT (mg/L)</th>
<th>TNT degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthine Oxidase</td>
<td>Pentolite</td>
<td>&lt;0.1</td>
<td>&gt;98.6%</td>
<td>&lt;0.1</td>
<td>&gt;99.3%</td>
</tr>
<tr>
<td>Control</td>
<td>Pentolite</td>
<td>6.9</td>
<td>0.0%</td>
<td>14</td>
<td>0.0%</td>
</tr>
<tr>
<td>Xanthine Oxidase</td>
<td>PETN</td>
<td>4.4</td>
<td>39.7%</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Control</td>
<td>PETN</td>
<td>7.3</td>
<td>0.0%</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
This significant enhancement of PETN degradation when presented to XO as Pentolite was reproducibly observed and is both significant and novel.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of deactivating an explosive composition provided in an explosive cartridge, which method comprises contacting the explosive composition with a deactivating agent that is capable of rendering the explosive composition insensitive to detonation and with another reagent that is capable of rendering the explosive composition insensitive to detonation, wherein the deactivating agent is an enzyme used in isolation from any living cell with which it might normally be associated or produced, wherein the another reagent is other than water, and wherein the deactivating agent and the another reagent render the explosive composition insensitive to detonation after a predetermined period of time.

2. A method according to claim 1, wherein the another reagent is selected from one or more of a microorganism, a non-biological chemical, a plant and a plant extract/derivative.

3. A method according to claims 1 or 2, wherein the enzyme is present in a laundry or dishwasher detergent.

4. A method according to claims 1 or 2, wherein the enzyme is provided with a cofactor.

5. A method according to claims 1 or 2, wherein the explosive composition is desensitised through the combined activity of deactivating agent and the another reagent and wherein the another reagent degrades the explosive composition into a by-product that is then degraded by the deactivating agent.

6. A method according to claims 1 or 2, wherein the explosive composition is desensitised through the combined activity of the deactivating agent and the another reagent and wherein the deactivating agent degrades the explosive composition into a by-product that is then degraded by the another reagent.
7. A method according to claims 5 or 6, wherein the another reagent is a reagent external to the explosive cartridge that will enter or be introduced into the cartridge during use thereof and that can contribute to desensitisation of the explosive composition.

8. A method according to claims 1 or 2, wherein the deactivating agent is provided in or in contact with the explosive composition, and wherein to effect desensitisation of the explosive composition the deactivating agent must be contacted with water that enters or is delivered into the cartridge when used.

9. A method according to claims 1 or 2, wherein the deactivating agent is provided separately from the explosive composition with the two coming into contact after a predetermined period of time.

10. A method according to claims 1 or 2, wherein the deactivating agent is provided separate to the explosive composition and must be mobilised in order for contact with the explosive composition to take place, and wherein the deactivating agent is provided in a suitable form that is rendered mobile by water that enters or is delivered into the explosive cartridge when used.

11. A method according to claim 10, wherein a water-permeable membrane separates the explosive composition and deactivating agent with the deactivating agent permeating this membrane when mobilised by contact with water.

12. A method according to claim 11, wherein the deactivating agent is provided in a water-degradable or water-soluble packet or wrapper.

13. A method according to claim 10, wherein the explosive cartridge includes one or more inlets and/or water-degradable pathways to allow environmental water to flow into the cartridge and directly into contact with the deactivating agent.

14. A method according to claims 10 or 13, wherein the explosive cartridge includes
one or more inlets and/or water-degradable pathways to allow environmental water to flow into the cartridge and into contact with the deactivating agent through the explosive composition.

15. A method according to claim 14, wherein the explosive composition includes channels to allow water to migrate to the deactivating agent.

16. A method according to claims 1 or 2, wherein the explosive cartridge takes the form of a seismic charge.

17. Use of a method according to claims 1 or 2 in a seismic survey application, wherein the explosive cartridge takes the form of a seismic charge.
Detonator in end-cap (5)

Poison reservoir (3)

Molded plastic outer shell

Emulsion explosive (2)

Axial cavity (4)

Possible second charge screw-coupled to first