

Report on the Deliberation Results

March 5, 2010

Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

[Brand name]	Moistear Ophthalmic Solution 3%
[Non-proprietary name]	Diquafosol Sodium (JAN*)
[Applicant]	Santen Pharmaceutical Co., Ltd.
[Date of application]	May 30, 2008

[Results of deliberation]

In the meeting held on February 26, 2010, the First Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product is not classified as a biological product or a specified biological product, the re-examination period is 8 years, and neither the drug substance nor the drug product is classified as a poisonous drug or a powerful drug.

“Diquas Ophthalmic Solution 3%” should replace “Moistear Ophthalmic Solution 3%” as the proposed brand name for Diquafosol Sodium.

**Japanese Accepted Name (modified INN)*

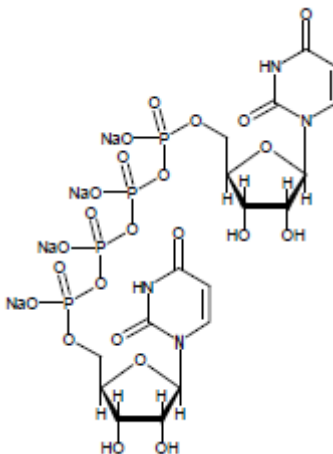
Review Report

February 9, 2010

Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name] Moistear Ophthalmic Solution 3%
[Non-proprietary name] Diquafosol Sodium
[Name of applicant] Santen Pharmaceutical Co., Ltd.
[Date of application] May 30, 2008
[Dosage form/Strength] An ophthalmic solution containing 30 mg of Diquafosol Sodium per mL
[Application classification] Prescription drug (1) Drug with a new active ingredient
[Chemical structure]



Molecular formula: $C_{18}H_{22}N_4Na_4O_{23}P_4$

Molecular weight: 878.23

Chemical name: Tetrasodium P^1, P^4 -bis (5'-uridyl) tetrphosphate

[Items warranting special mention] None

[Reviewing office] Office of New Drug IV

This English version of the Japanese review report is intended to be a reference material to provide convenience for users. In the event of inconsistency between the Japanese original and this English translation, the former shall prevail. The PMDA will not be responsible for any consequence resulting from the use of this English version.

Review Results

February 9, 2010

[Brand name] Moistear Ophthalmic Solution 3%
[Non-proprietary name] Diquafosol Sodium
[Name of applicant] Santen Pharmaceutical Co., Ltd.
[Date of application] May 30, 2008

[Results of review]

Based on the submitted data, it is concluded that the efficacy of the product in dry eye treatment has been demonstrated and its safety is acceptable in view of its observed benefits. Although it is considered that there are no particular safety problems at present, it is necessary to continue to investigate the safety of the product in routine clinical settings via post-marketing surveillance.

As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved for the following indication and dosage and administration.

[Indication]

Dry eye

[Dosage and administration]

The usual dose is one drop instilled into the eye six times a day.

Review Report (1)

January 13, 2010

I. Product Submitted for Registration

[Brand name]	Moistear Ophthalmic Solution 3%
[Non-proprietary name]	Diquafosol Sodium
[Name of applicant]	Santen Pharmaceutical Co., Ltd.
[Date of application]	May 30, 2008
[Dosage form/Strength]	An ophthalmic solution containing 30 mg of Diquafosol Sodium per mL
[Proposed indication]	Corneal and conjunctival epithelial damage associated with dry eye (including Sjogren's syndrome and Stevens-Johnson syndrome)
[Proposed dosage and administration]	The usual dose is one drop instilled into the eye six times a day.
[Items warranting special mention]	None

II. Summary of the Submitted Data and Outline of Review by Pharmaceuticals and Medical Devices Agency

A summary of the submitted data and an outline of the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

1. Origin or history of discovery and usage conditions in foreign countries etc.

The active ingredient of the product, Diquafosol Sodium (diquafosol), is a dinucleotide which functions as an agonist at the P2Y₂ purinergic receptor expressed in the conjunctiva and lungs (mucosal tissues) and its pharmacological effects were discovered by Inspire Pharmaceuticals, Inc. (the US).

Dry eye is a chronic multifactorial disease of the tears and corneal and conjunctival epithelium that results in symptoms of ocular discomfort and visual disturbance. Currently, there is no curative treatment for dry eye and artificial tears and sodium hyaluronate (HA) ophthalmic solution have been used as pharmacotherapies for dry eye and for patients with an inadequate response to currently available eye drops, autologous serum eye drop treatment or surgical procedures such as punctal plug insertion and punctal occlusion have been used. As it was considered that diquafosol acts on the P2Y₂ receptor and promotes fluid and mucin secretion, contributing to an improvement in the quantity and quality of tears, the drug was developed as an ophthalmic solution for dry eye with a novel mechanism of action.

In Japan, clinical development for diquafosol was initiated in ■■■ 20■■■. The applicant claims that the

efficacy and safety of diquafosol in dry eye treatment have been confirmed in Japanese clinical studies, and a marketing application for diquafosol has been filed.

Outside Japan, diquafosol is not approved or being developed in any country at present. However, an ophthalmic solution containing diquafosol as the active ingredient (a different formulation of diquafosol) developed by Inspire Pharmaceuticals, Inc. (the US) is under review in the US as of November 2009.

2. Data relating to quality

2.A Summary of the submitted data

Yamasa Corporation has registered a drug master file for Diquafosol Sodium, i.e. the drug substance of the product (DMF No. 220MF10109). The summary of the submitted data and the outline of review pertaining to the manufacturing process for the drug substance, etc. are as shown in the Appendix.

2.A.(1) Drug product

The drug product is an aqueous ophthalmic solution containing 3% of the drug substance supplied in a multi-dose bottle. Other than the drug substance, it contains a buffer agent, isotonic agents, a preservative (benzalkonium chloride), a pH adjuster, and a solvent. The container closure system for the drug product is a polyethylene bottle with a polypropylene cap. All of the excipients are those listed in the Japanese Pharmacopoeia and no novel excipient is used.

The manufacturing process for the drug product consists of Step 1 (), Step 2 (sterile filtration,), and Step 3 (packaging and labeling). Step has been defined as a critical step and in-process tests and action limits have been established for pH, sterile filter integrity, , and mass.

The proposed specifications for the drug product are description, identification (UV-visible absorption spectrum), osmotic pressure ratio, pH, foreign insoluble matter, insoluble particulate matter, sterility, the content of benzalkonium chloride (HPLC), and assay (HPLC). Purity tests (related substances [, , , , , unknown related substances]) have been performed, but are not included in the specification since no new degradation products formed specifically in the drug product during formulation development and all of these related substances were at levels up to % in long-term and accelerated stability studies.

In order to assess the stability of the drug product, long-term testing (25°C/40%RH/dark place/24 months), accelerated testing (40°C/not more than 25%RH/dark place/6 months), stress testing (light [25°C/ambient humidity/1.2 million lx-hr + 226 W-h/m²]), and stress testing (temperature [60°C/ambient humidity/dark place/2 months]) were performed. In addition to the drug product specification tests, purity (related substances), water loss, and preservative effectiveness tests were

conducted.

Under the long-term storage condition, decreased pH (about [REDACTED]) and increases in the osmotic pressure ratio (about [REDACTED]) and the assay of Diquafosol Sodium and the content of benzalkonium chloride (about [REDACTED]% each) associated with water loss ([REDACTED]%-[REDACTED]%) were observed. Also under the accelerated condition, decreased pH (about [REDACTED]) and increases in the osmotic pressure ratio (about [REDACTED]), the assay of Diquafosol Sodium (about [REDACTED]%), and the content of benzalkonium chloride (about [REDACTED]%) associated with water loss ([REDACTED]%-[REDACTED]%) were observed. Under the stress condition (light), there were no changes in any of the attributes tested. Under the stress condition (temperature), decreased pH, increases in the osmotic pressure ratio, the assay of Diquafosol Sodium, and the content of benzalkonium chloride associated with water loss, and a trend towards increases in the levels of related substances were observed. As the changes with time observed in the long-term and accelerated stability studies were considered within acceptable ranges, a shelf life of 24 months has been proposed for the drug product when stored at room temperature. The long-term storage study is ongoing up to 36 months.

2.B Outline of the review by PMDA

2.B.(1) Drug product

As the product is intended to be instilled into the eye six times a day, PMDA asked the applicant to explain the effects of decreasing remaining drug solution during use on its stability.

The applicant explained as follows:

As one bottle of Moistear Ophthalmic Solution 3% will last for about 10 to 12 days after opening the bottle of the drug product, one drop was instilled 6 times daily for 12 days in order to simulate the usage of the product. The attributes tested before and after use were description, osmotic pressure ratio, pH, related substances, foreign insoluble matter, the content of benzalkonium chloride, and the assay of Diquafosol Sodium. As a result, there were no particular changes in any of the attributes tested and the drug product after use was also tested for total viable count and showed no microbial growth. Therefore, there should be no problem with the in-use stability of the drug product.

PMDA accepted the above response and concluded that the proposed specifications, test procedures, storage conditions, and shelf life for the drug product are acceptable.

3. Non-clinical data

3.(i) Summary of pharmacology studies

3.(i).A Summary of the submitted data

The results from the following primary pharmacodynamic studies of diquafosol were submitted: studies on stimulation of tear and mucin secretion using a normal or dry eye animal model; studies on the improvement and prevention of corneal epithelium damage using a dry eye animal model; a comparative study of the effect of diquafosol and currently available HA ophthalmic solution using a

dry eye animal model; and studies on the mechanism of action of diquafosol. As diquafosol meets “the conditions under which studies are not necessary” as specified in the Guideline for Safety Pharmacology Studies for Human Pharmaceuticals, a GLP-compliant safety pharmacology study based on the current guideline was not performed. However, the results from non-GLP general pharmacology studies were submitted as reference data. No secondary pharmacodynamic or pharmacodynamic drug interaction studies have been conducted.

3.(i).A.(1) Primary pharmacodynamics

3.(i).A.(1).1 Effects in normal animals

(a) Stimulation of tear secretion in normal rabbits

i) Single-dose effects (4.2.1.1-001, 4.2.1.1-002)

Male rabbits received a single instillation of 50 µL of 8.5% diquafosol in both eyes and tear secretion was measured by Schirmer’s test. Schirmer’s test scores at 5, 15, and 30 minutes after instillation were significantly higher with diquafosol than with saline with a maximal score at 15 minutes after instillation. Following a single ocular instillation of 0.1%, 0.3%, 1%, 3%, or 8.5% diquafosol, a concentration-dependent increase in Schirmer’s test score at 15 minutes after instillation was observed, significant differences from saline were seen at concentrations of $\geq 0.3\%$, and its maximal effect was reached at concentrations of $\geq 1\%$.

ii) Repeat-dose effects (4.2.1.1-003)

Male rabbits received 50 µL of 0.3%, 1%, or 3% diquafosol in both eyes 6 times daily for 4 weeks. Schirmer’s test scores at 15 minutes after instillation were significantly higher with all concentrations of diquafosol than with saline throughout the 4-week dosing period.

(b) Increase in tear proteins in normal rabbits (4.2.1.1-004)

Male rabbits received a single instillation of 50 µL of 0.3%, 1%, or 3% diquafosol in both eyes. A concentration-dependent increase in Schirmer’s test score was observed and significant differences from vehicle were seen at all concentrations of diquafosol. Proteins extracted from filter paper strips were quantified by Bradford assay. The tear protein concentrations in all diquafosol groups were similar to that in the vehicle group (about 30 mg/mL) and there were no significant differences. Meanwhile, a concentration-dependent increase in the absolute protein content calculated by multiplying tear protein concentration by tear volume was observed and significant differences from vehicle were seen at concentrations of 1% and 3%.

(c) Stimulation of tear secretion in normal rats (4.2.1.1-005, 4.2.1.1-006)

Male rats received a single instillation of 5 µL of 8.5% diquafosol in both eyes. Schirmer’s test scores at 10 and 30 minutes after instillation were significantly higher with diquafosol than with saline with a maximal score at 10 minutes after instillation. Following a single ocular instillation of 0.1%, 0.3%, 1%, 3%, or 8.5% diquafosol, Schirmer’s test scores at 10 minutes after instillation were significantly higher with $\geq 3\%$ diquafosol than with saline.

(d) Stimulation of mucin secretion from conjunctival goblet cells in normal rabbits (4.2.1.1-007, 4.2.1.1-008)

Male rabbits received a single instillation of 50 μL of 8.5% diquafosol in both eyes and then impression cytology was performed. The percentage of PAS-positively stained area per mm^2 of conjunctival epithelial tissue (cellulose acetate filters) (%) was calculated as an index of mucin-like glycoprotein content in conjunctival goblet cells and mucin secretion from conjunctival goblet cells was expressed as a reduction in the percentage of PAS-positively stained area. The percentage of PAS-positively stained area was significantly reduced at 2, 5, and 15 minutes after the instillation of diquafosol compared with saline instillation. Following a single ocular instillation of 0.001%, 0.01%, 0.1%, 1%, or 8.5% diquafosol, impression cytology was performed at 5 minutes post-instillation. The percentage of PAS-positively stained area was significantly reduced in the $\geq 0.01\%$ diquafosol groups compared with the saline group with a maximal reduction at concentrations of $\geq 0.1\%$.

3.(i).A.(1).2) Effects in dry eye animal model

(a) Stimulation of tear secretion in a rat model of dry eye developed by surgically removing the exorbital lacrimal gland (4.2.1.1-009)

Male rats with the exorbital lacrimal glands removed received a single instillation of 5 μL of 0.1%, 0.3%, 1%, 3%, or 8.5% diquafosol in both eyes. In untreated rats with the exorbital lacrimal glands removed, Schirmer's test scores were significantly decreased to about a half of those in normal rats. Diquafosol concentration-dependently increased the Schirmer's test score in rats with the exorbital lacrimal glands removed and significant differences from saline were seen at concentrations of 3% and 8.5%.

(b) Increase in conjunctival epithelial mucins in a rat model of dry eye developed by surgically removing the exorbital lacrimal gland (4.2.1.1-010)

Male rats with the exorbital lacrimal glands removed received 5 μL of 0.3%, 1%, or 3% diquafosol in both eyes 6 times daily for 4 weeks and then conjunctival tissue sections were prepared, and the PAS-positively stained area (mm^2) was calculated as an index of mucin-like glycoprotein content in conjunctival goblet cells. In untreated rats with the exorbital lacrimal glands removed, the PAS-positively stained areas were significantly reduced to about 65% of those in normal rats. Diquafosol concentration-dependently increased the PAS-positively stained area in rats with the exorbital lacrimal glands removed and significant differences from vehicle were seen at concentrations of 1% and 3%.

(c) Improvement of corneal epithelial damage in a rat model of dry eye developed by surgically removing the exorbital lacrimal gland

i) Dose response study (4.2.1.1-011)

Male rats with the exorbital lacrimal glands removed received 5 μL of 0.03%, 0.1%, 0.3%, 1%, or 3% diquafosol in both eyes 6 times daily for 4 weeks. In untreated rats with the exorbital lacrimal glands removed, the corneal permeability to fluorescein as an index of the degree of corneal epithelial

damage was about 3.5-fold higher than that in normal rats. Treatment with diquafosol resulted in a concentration-dependent decrease in the permeability to fluorescein in rats with the exorbital lacrimal glands removed. Diquafosol significantly reduced the permeability of fluorescein at a concentration of 3% at Week 1 and at concentrations of $\geq 0.1\%$ at Weeks 2 and 4 compared with saline and at Week 4, a maximal effect was reached at concentrations of $\geq 1\%$.

ii) Determination of frequency of instillation (4.2.1.1-012)

Male rats with the exorbital lacrimal glands removed received 5 μL of 1% diquafosol in both eyes 2, 4, 6, or 8 times a day for 2 weeks. In untreated rats with the exorbital lacrimal glands removed, the corneal permeability to fluorescein was about 3.5-fold higher than that in normal rats. Diquafosol reduced the permeability of fluorescein in a daily instillation frequency-dependent manner in rats with the exorbital lacrimal glands removed. Diquafosol instilled ≥ 4 times a day significantly reduced the permeability to fluorescein compared with saline and a maximal effect was reached with the instillation frequency of ≥ 6 times a day.

(d) Prevention of corneal epithelial damage in a rabbit model of desiccation-induced dry eye (4.2.1.1-013, 4.2.1.1-014)

Male rabbits received a single instillation of 50 μL of 1% diquafosol in both eyes and then their both eyes were held open with a speculum for 3 hours. The absorbance of methylene blue as an index of the degree of corneal epithelial damage was increased with the duration of desiccation in the saline group while little increase in the absorbance was observed in the diquafosol group. The absorbance was significantly lower in the diquafosol group compared with the saline group at all desiccation times. Following a single ocular instillation of 0.001%, 0.01%, 0.1%, or 1% diquafosol, a concentration-dependent reduction in the absorbance of methylene blue after 3 hours of desiccation was observed and significant differences from saline were seen at concentrations of $\geq 0.1\%$.

(e) Comparison of the effect of diquafosol vs. 0.1% HA ophthalmic solution on corneal epithelial damage in a rat model of dry eye developed by surgically removing the exorbital lacrimal gland (4.2.1.1-015)

Male rats with the exorbital lacrimal glands removed received 5 μL of 1% or 3% diquafosol or 0.1% HA in both eyes 6 times daily for 6 weeks. Fluorescein corneal staining scores at all timepoints were significantly higher in untreated rats with the exorbital lacrimal glands removed than in normal rats. All of 1% and 3% diquafosol and 0.1% HA reduced corneal staining scores in rats with the exorbital lacrimal glands removed, showing significant differences from vehicle at Weeks 4 and 6. At Week 6, the corneal staining score was significantly lower in the 3% diquafosol group than in the 0.1% HA group.

3.(i).A.(1).3 Mechanism of action

(a) Inositol trisphosphate formation in cells expressing P2Y receptors (4.2.1.1-016)

In 1321N1 cells expressing the human P2Y receptor subtypes (P2Y₁, P2Y₂, P2Y₄, or P2Y₆), the

affinity of diquafosol at each P2Y receptor subtype was determined by inositol trisphosphate formation. In cells expressing the P2Y₂ receptor, the EC₅₀ value of diquafosol for inositol trisphosphate formation was 0.15 μM, which was comparable to the EC₅₀ value of the positive control, UTP (0.17 μM). In cells expressing the P2Y receptor subtypes, the maximal response to the positive control was set at 100% to compare the EC₅₀ values of diquafosol for the different receptor subtypes. As a result, the rank order of the affinity of diquafosol was: P2Y₂ = P2Y₄ > P2Y₆ > P2Y₁.

(b) Affinity for various membrane receptors and ion channels (4.2.1.1-017)

Using membrane preparations from tissues expressing various receptors and ion channels or cells forcibly expressing these receptors and ion channels, the inhibitory effects of 10⁻⁵ M of diquafosol on labeled ligand binding to the receptors and ion channels were assessed. Diquafosol did not cause ≥50% inhibition of ligand binding to any receptor other than the P2Y receptors or any ion channel.

(c) Localization of P2Y₂ receptors in conjunctival tissue (4.2.1.1-018)

The localization of the P2Y₂ receptor in the palpebral/bulbar tissues from a rhesus monkey was assessed by *in situ* hybridization using digoxigenin-labeled P2Y₂ receptor riboprobes. In the lung/bronchial tissues as a positive control, P2Y₂ receptor expression was localized to the bronchial epithelium (including goblet cells) and submucosal gland epithelium. In the palpebral/bulbar tissues, P2Y₂ receptor expression was localized to the palpebral/bulbar conjunctival epithelium (including goblet cells) and meibomian gland (adipocytes and duct epithelium).

(d) Increases in intracellular calcium ion concentration in rabbit conjunctival epithelial cells (4.2.1.1-019)

The effect of 1 to 1000 μM of diquafosol on intracellular calcium ion concentration in cultured rabbit conjunctival epithelial cells was determined by Fura-2 double wavelength (340 nm/380 nm) fluorometry. Diquafosol at ≥10 μM significantly increased intracellular calcium ion concentration compared with vehicle, showing an almost maximal response at 100 μM.

(e) Stimulation of Cl ion transport in excised rabbit conjunctival tissue (4.2.1.1-020, 4.2.1.1-021)

Using excised rabbit conjunctival tissues, the effect of diquafosol on Cl ion transport was investigated by measuring short-circuit current. When 10⁻⁷ to 10⁻³ M of diquafosol or UTP was added to the tear (epithelial) side, the short-circuit current transiently increased immediately after addition and then decreased gradually. The respective percent increases in the short-circuit current above the control levels were concentration-dependent with an almost maximal response both at 10⁻⁵ M (10 μM) of diquafosol and UTP.

Then, the effect of a calcium chelator, BAPTA-AM, on the increase in the short-circuit current induced by diquafosol was investigated. The increase in the short-circuit current induced by the addition of 10 μM diquafosol to excised rabbit conjunctival tissue was reduced by pretreatment with BAPTA-AM (3, 10, 30 μM) in a concentration-dependent manner and significant differences from no treatment were

seen at concentrations of $\geq 10 \mu\text{M}$.

(f) Stimulation of fluid secretion in excised rabbit conjunctival tissue (4.2.1.1-020)

Using excised rabbit conjunctival tissues, the effect of diquafosol on fluid transport was investigated. Addition of 10^{-5} M diquafosol or 10^{-5} M UTP to the tear (epithelial) side increased the rate of stromal (basolateral)-to-tear transport of fluid by about 50%.

(g) Stimulation of mucin secretion in excised rabbit conjunctival tissue (4.2.1.1-022 to 4.2.1.1-024)

Using excised rabbit conjunctival tissues, the effect of diquafosol on the secretion of mucin-like glycoproteins was investigated. When the excised rabbit conjunctival tissues were incubated with $100 \mu\text{M}$ diquafosol for 30 to 120 minutes, the mucin-like glycoprotein content of incubation medium was increased in an incubation time-dependent manner and significant differences from vehicle were seen at all incubation times. When the excised rabbit conjunctival tissues were incubated with 1 to $1000 \mu\text{M}$ of diquafosol for 90 minutes, the mucin-like glycoprotein content of incubation medium was increased in a diquafosol concentration-dependent manner and significant differences from vehicle were seen at concentrations of $\geq 10 \mu\text{M}$ and a maximal response was reached at $\geq 100 \mu\text{M}$.

Then, the effect of a calcium chelator, BAPTA-AM, on the stimulation of mucin-like glycoprotein secretion with diquafosol was investigated. When excised rabbit conjunctival tissues were incubated with $100 \mu\text{M}$ diquafosol for 90 minutes, the mucin-like glycoprotein content of incubation medium was about 2.7-fold higher than that in the vehicle group, but this effect was suppressed by pretreatment with BAPTA-AM (3, 10, $30 \mu\text{M}$) in a concentration-dependent manner and significant differences from no treatment were seen at concentrations of $\geq 10 \mu\text{M}$.

Based on the above, the applicant explained that diquafosol is considered to activate the P2Y_2 receptors in conjunctival epithelium and goblet cell membranes, and increase intracellular calcium ion concentration, thereby stimulating fluid secretion driven by Cl ion transport and mucin secretion.

3.(i).A.(2) Safety pharmacology (Reference data 4.2.1.3-001)

3.(i).A.(2).1) Effects on central nervous system

Following intravenous administration of diquafosol to male mice ($n = 6-10$ per group), there were no treatment-related effects on general symptoms and behavior, spontaneous locomotor activity, hexobarbital-induced anesthesia, electroshock seizures, or pentylenetetrazol-induced seizures at doses up to 100 mg/kg . Following intravenous administration of 1, 10, or 100 mg/kg of diquafosol to male rats ($n = 10$ per group), the effects of diquafosol on nociception and body temperature were assessed. Diquafosol did not affect pain reaction at any dose level and had no effect on body temperature up to 10 mg/kg . However, at 100 mg/kg , body temperature decreased slightly at 30 minutes post-dose, which resolved by 120 minutes post-dose.

3.(i).A.(2).2) Effects on autonomic nervous system and smooth muscle

Diquafosol had no effects on the spontaneous contractions of rabbit isolated ileum at concentrations up to 10^{-6} M, but tended to inhibit the spontaneous contractions at 10^{-5} M and significantly inhibited the spontaneous contractions at 10^{-4} M. Diquafosol at concentrations up to 10^{-4} M had no effect on the tension of isolated guinea pig trachea.

3.(i).A.(2).3) Effects on respiratory/cardiovascular system

Diquafosol was administered as a continuous intravenous infusion at doses of 10, 30, and 100 mg/kg/30 min to anesthetized dogs (4 males and 4 females). No changes in respiration were observed. Heart rate and left ventricular pressure (LVP) tended to decrease, although not significant. A continuous infusion of diquafosol at 30 or 100 mg/kg/30 min significantly decreased the mean blood pressure. A continuous infusion of diquafosol at 100 mg/kg/30 min significantly reduced LVdP/dt, i.e. the differential of LVP and femoral artery blood flow. An ECG showed QT interval prolongation at all dose levels, but no changes in QTc were observed. A continuous infusion of diquafosol at 100 mg/kg/30 min prolonged the PR interval. These series of changes were observed only during continuous infusion and resolved quickly after the end of infusion.

3.(i).A.(2).4) Effects on water and electrolyte metabolism

Diquafosol at doses of 1, 10, and 100 mg/kg was intravenously administered to male rats (n = 6 per group). Although no effects on urine volume or urinary electrolyte excretion were observed at doses up to 10 mg/kg, there were significant reductions in urine volume and urinary Na^+ and Cl^- excretion at 100 mg/kg.

3.(i).B Outline of the review by PMDA

The rank order of the affinity of diquafosol for different P2Y receptor subtypes was $\text{P2Y}_2 = \text{P2Y}_4 > \text{P2Y}_6 > \text{P2Y}_1$ and diquafosol has affinity not only for the P2Y_2 receptor but also for other subtypes. PMDA asked the applicant to discuss the potential of diquafosol to cause unintended effects via these receptors after topical ocular application, taking account of the distribution and physiological functions of each P2Y receptor subtype in humans.

The applicant explained as follows:

The P2Y_1 , P2Y_2 , P2Y_4 , and P2Y_6 receptors are all distributed extensively throughout the body and have various physiological functions (von Kügelgen I. *Pharmacol Ther.* 2006;110: 415-32, Erlinge D, Burnstock G. *Purinergic Signal.* 2008;4: 1-20, Fischer W, Krügel U. *Curr Med Chem.* 2007;14: 2429-55). However, diquafosol was not detectable in plasma and its endogenous metabolites also did not exceed their physiological concentrations after topical ocular administration in humans. Therefore, it is highly unlikely that diquafosol is systemically absorbed and affects the homeostasis of the body after topical ocular administration. In addition, since it has been reported that the P2Y_1 , P2Y_2 , P2Y_4 , and P2Y_6 receptors are present in corneal epithelial cells on the human ocular surface and play a role in corneal epithelial injury repair process (Klepeis VE et al. *J Cell Biochem.* 2004;93: 1115-33), it is

inferred that activation of any of these receptors by diquafosol results in improvement in corneal epithelial damage. There are no reports on the expression and physiological functions of the P2Y₁, P2Y₄, and P2Y₆ receptors in other human ocular surface tissues, except for a report that conjunctival cells also contain P2Y₂ receptors (Jumblatt JE, Jumblatt MM. *Exp Eye Res.* 1998;67: 341-6). Thus, whether diquafosol causes unintended effects in these tissues is unknown. In human ocular tissues other than the ocular surface tissues, the expression of P2Y receptors in trabecular meshwork cells (P2Y₁, P2Y₄) (Crosson CE et al. *J Pharmacol Exp Ther.* 2004;309: 484-9), retinal pigment epithelial cells (P2Y₁, P2Y₂, P2Y₄) (Tovell VE, Sanderson J. *Invest Ophthalmol Vis Sci.* 2008;49: 350-7), and retinal Muller cells (P2Y₁, P2Y₂, P2Y₄, P2Y₆) (Fries JE et al. *Invest Ophthalmol Vis Sci.* 2005;46: 3000-7) has been reported. However, diquafosol is rapidly metabolized to uridine and uracil on the ocular surface after topical ocular application and these metabolites show little affinity for the P2Y₁, P2Y₂, P2Y₄, and P2Y₆ receptors (4.2.2.4-002, 4.2.2.4-004). For these reasons, the P2Y₁, P2Y₂, P2Y₄, and P2Y₆ receptors in ocular tissues other than the ocular surface tissues should be unaffected.

PMDA accepted the above response and concluded from the submitted data and responses that diquafosol ophthalmic solution for dry eye has pharmacological significance.

3.(ii) Summary of pharmacokinetic studies

3.(ii).A Summary of the submitted data

As absorption, distribution, metabolism, and excretion data, the results from single-instillation or single intravenous administration studies in rats and rabbits were submitted. The results from toxicokinetic studies by repeated instillation in rabbits and repeated intravenous administration to rats and dogs were submitted. Diquafosol and radiolabeled diquafosol (¹⁴C-diquafosol) were used for pharmacokinetic studies, and plasma concentrations of diquafosol and its metabolites were determined using high performance liquid chromatography with UV detection (HPLC-UV) or high performance liquid chromatograph-tandem mass spectrometry (LC-MS/MS) (Lower limit of quantification for plasma diquafosol, 100 ng/mL or 2 ng/mL). Tissue radioactivity was determined with a liquid scintillation counter (Lower limit of quantification, 2 times the background radioactivity or 30 cpm) and the metabolites of ¹⁴C-diquafosol were determined using radio-HPLC (Lower limit of quantification, 2 or 3 times the background radioactivity). Unless otherwise specified, pharmacokinetic parameters are expressed as the mean or the mean ± standard deviation (SD).

3.(ii).A.(1) Absorption

3.(ii).A.(1).1 Single-dose studies (4.2.2.2-001 to 4.2.2.2-003)

Following a single instillation of 3% ¹⁴C-diquafosol into both eyes of rats (6 males and 6 females) (5 µL in each eye; 0.3 mg/rat; mean dose, 1.1 mg/kg in males, 1.6 mg/kg in females), the pharmacokinetic parameters in males and females were as follows: the C_{max} was 170 ± 91 and 267 ± 100 ng eq./mL, respectively; the t_{max} was 1 and 0.5 hours, respectively; the AUC_{0-24hr} was 859 and 1090 ng eq.·hr/mL, respectively; and the t_{1/2} was 16 and 15 hours, respectively.

Following a single intravenous administration of 1 mg/kg of ^{14}C -diquafosol to male rats ($n = 4$), the $\text{AUC}_{0-24\text{hr}}$ was 1050 ng eq.·hr/mL and the $t_{1/2}$ was 12 hours. The bioavailability of ocularly instilled diquafosol calculated using the $\text{AUC}_{0-24\text{hr}}$ value of intravenous diquafosol was 81.8%.

Following a single instillation of 3% ^{14}C -diquafosol into both eyes of male rabbits ($n = 2$) (50 μL in each eye; 3 mg/rabbit; 1.36 mg/kg), the C_{max} was 354.8 ng eq./mL, the t_{max} was 0.5 hours, the $\text{AUC}_{0-\infty}$ was 2551.2 ng eq.·hr/mL, and the $t_{1/2}$ was 17.4 hours.

3.(ii).A.(1).2 Repeat-dose studies (toxicokinetic studies) (4.2.3.2-001 to 4.2.3.2-006)

Rabbits (5 rabbits/sex/group) received 50 μL of 8% diquafosol or placebo in one eye 7 times daily for 6 weeks and plasma concentrations of diquafosol and its metabolites (UTP, UDP, UMP) were measured on Day 1 and at Week 6 (before instillation [at Week 6 only] and 5, 15, 30, and 60 minutes after instillation). On Day 1, diquafosol was below the lower limit of quantification (2 ng/mL), except at 5 or 30 minutes after instillation in 3 of 10 rabbits (2.03-3.93 ng/mL). At Week 6, diquafosol was quantifiable before instillation in 5 rabbits (2.13-4.60 ng/mL) and after instillation, diquafosol was quantifiable sporadically regardless of the sampling time in 9 rabbits (2.13-5.41 ng/mL). Also in the placebo group, diquafosol was below the lower limit of quantification in all 10 rabbits on Day 1, but was quantifiable (2.32-4.44 ng/mL) in 6 rabbits at Week 6. Although UTP was quantifiable in all 10 rabbits on Day 1 and in 7 rabbits at Week 6, its concentrations were 100.52 to 316.80 ng/mL, i.e., near the lower limit of quantification (100 ng/mL), and similar levels of UTP were detected even before instillation or in the placebo group. UDP and UMP were below the lower limit of quantification (100 ng/mL) at most of the sampling points in both the diquafosol and placebo groups.

Rabbits (6 rabbits/sex/group) received 50 μL of 8% diquafosol or placebo in one eye 7 times daily for 9 months and plasma concentrations of diquafosol and its metabolites (uridine, uracil) were measured on Day 1 and at Weeks 6, 13, and 39 (before the 1st instillation and 5-60 minutes after the 7th instillation on each measurement day [before the 1st instillation and 5 minutes after the 7th instillation on each measurement day for diquafosol; all sampling points on each measurement day for uridine; and all sampling points at Weeks 13 and 39 for uracil]). Diquafosol was below the lower limit of quantification (2 ng/mL), except before instillation (3.43-18.05 ng/mL in 5 of 12 rabbits) and at 5 minutes after the 7th instillation (2.80 ng/mL in 1 of 12 rabbits) at Week 13. Uridine and uracil were also quantifiable after topical ocular instillation of diquafosol, but their concentrations did not change over time or with repeated instillations, and similar levels of uridine and uracil were detected even before instillation or in the placebo group.

Based on the above, the applicant explained as follows:

Although UTP, UDP, UMP, uridine, and uracil were quantifiable after topical ocular instillation of diquafosol in rabbits, as similar levels of UTP, UDP, UMP, uridine, and uracil were detected even before instillation or in the placebo group, it seems that the majority of these substances were those produced by endogenous processes. Therefore, the systemic exposures to unchanged diquafosol and

metabolites resulting from topical ocular instillation of diquafosol should be extremely low.

Rats (10 rats/sex/group) were intravenously administered 10, 30, or 100 mg/kg/day of diquafosol for 1 month and plasma concentrations of diquafosol and its metabolites (UTP, UDP, UMP) were measured on Day 1 and at Week 4. Diquafosol was below the lower limit of quantification (100 ng/mL)¹ at all sampling points. UTP was quantifiable in the 10 mg/kg and 100 mg/kg groups, but was below the lower limit of quantification (100 ng/mL) at all sampling points in the 30 mg/kg group. UDP was below the lower limit of quantification (100 ng/mL), except at 5 minutes post-dose on Day 1 in the 100 mg/kg group. UMP was below the lower limit of quantification (500 ng/mL) at all sampling points.

Rats (9 rats/sex/group) were intravenously administered 3, 10, 30, or 100 mg/kg/day of diquafosol for 6 months and plasma concentrations of diquafosol and its metabolites (UTP, UDP, UMP, uridine) were measured on Days 1 and 29 and at Weeks 13 and 26. Diquafosol was below the lower limit of quantification (100 ng/mL) at all sampling points. Although UTP was quantifiable regardless of the dose and sampling point, its concentrations were near the lower limit of quantification (100 ng/mL). UDP and UMP were below the lower limit of quantification (100 and 500 ng/mL, respectively) at most of the sampling points. Uridine was quantifiable at 2 (at the first sampling point) to 15 minutes post-dose and fell below the lower limit of quantification (500 ng/mL) by 30 minutes post-dose at ≥ 10 mg/kg/day. Although more than dose-proportional increases in uridine concentration were observed at 2 minutes post-dose, there were no major changes with repeated administration at all dose levels.

Dogs (3 dogs/sex/group) were intravenously administered 50, 100, or 150 mg/kg/day of diquafosol for 1 month, and plasma concentrations of diquafosol and its metabolites (UTP, UDP, UMP) were measured on Day 17 and at Week 4. In the 100 and 150 mg/kg/day groups, diquafosol was quantifiable at 5 minutes post-dose (at the first sampling point) and fell below the lower limit of quantification (100 ng/mL) by 30 minutes post-dose. UTP and UMP at 100 and 150 mg/kg and UDP at 150 mg/kg were quantifiable at 5 minutes post-dose and fell below the lower limit of quantification (100 ng/mL for UTP and UDP; 500 ng/mL for UMP) by 30 minutes post-dose.

Dogs (4 dogs/sex/group) were intravenously administered 15, 50, or 150 mg/kg/day of diquafosol for 9 months and plasma concentrations of diquafosol and its metabolites (UTP, UDP, UMP, uridine) were measured on Day 1 and at Weeks 4, 13, 26, and 39. Diquafosol was below the lower limit of quantification (100 ng/mL) at all sampling points in the 15 and 50 mg/kg groups. Diquafosol was quantifiable at 5 (at the first sampling point) to 15 minutes post-dose and fell below the lower limit of quantification by 30 minutes post-dose in the 150 mg/kg group. In the 150 mg/kg group, UTP, UDP, and UMP were quantifiable at 5 minutes to 1 hour post-dose and fell below the lower limit of

¹ In repeated intravenous administration studies in rats and dogs (4.2.3.2-003 to 4.2.3.2-006), at each sampling point, the concentration was reported as below the lower limit of quantification if at least half of the samples were below the lower limit of quantification or the mean of the samples was below the lower limit of quantification.

quantification (100 ng/mL for UTP and UDP; 500 ng/mL for UMP) by 2 hours post-dose. Uridine was quantifiable at 5 minutes post-dose at all dose levels and fell below the lower limit of quantification (200 ng/mL) by 15 minutes post-dose in the 15 mg/kg group, by 30 minutes post-dose in the 50 mg/kg group, and by 2 hours post-dose in the 150 mg/kg group. Although more than dose-proportional increases in uridine concentration were observed at 5 minutes post-dose, there were no major changes with repeated administration at all dose levels.

The applicant explained the reason for quantifiable levels of plasma diquafosol even before the ocular instillation of diquafosol and in the placebo group in a rabbit repeat-dose study as follows:

Blank plasma samples used for validation showed a small peak that would not interfere with the assay near the retention time of unchanged diquafosol. Therefore, there is a possibility that in some samples, the peak response of an endogenous contaminant exceeded the peak response of unchanged diquafosol at the lower limit of quantification and the endogenous contaminant was falsely quantified as unchanged diquafosol.

PMDA concluded as follows:

Although the specificity of the assay for rabbit plasma is still questionable, the conclusion that plasma concentrations of diquafosol (unchanged diquafosol) are low remains unchanged. Thus, it is possible to evaluate the results of this study.

3.(ii).A.(2) Distribution

3.(ii).A.(2).1 Ocular and systemic tissue distribution studies (4.2.2.2-001 to 4.2.2.2-003)

Following a single instillation of 3% ¹⁴C-diquafosol into both eyes of rats (6 males and 6 females) (5 µL in each eye, 0.3 mg/rat), radioactivity was distributed extensively into ocular and systemic tissues. Among ocular tissues, high levels of radioactivity were present in external ocular tissues and the level of radioactivity was highest in the cornea at 4 hours post-instillation (males, 5665 ± 5451 ng eq./g; females, 4259 ± 1880 ng eq./g) followed by the bulbar conjunctiva, iris-ciliary body, and then aqueous humor at 15 minutes post-instillation (3288 ± 1614, 3180 ± 1262, and 1872 ± 208 ng eq./mL, respectively, in male rats). Among systemic tissues, the levels of radioactivity were highest in the duodenum at 1 hour post-instillation (males, 653 ± 434 ng eq./g; females, 941 ± 725 ng eq./g), which were 3.84-fold and 4.28-fold higher than the plasma levels in males and females, respectively. Elimination of radioactivity from the ocular and systemic tissues was slow. Whole-body autoradioluminography in rats (2 males and 2 females) showed that radioactivity was present in the eyeballs at 10 minutes post-instillation and in the stomach, intestinal content, and oral cavity and near the esophagus at 1 hour post-instillation, indicating that some of diquafosol or its metabolites are distributed into the gastrointestinal tract after topical ocular instillation. The radioactivity distribution in ocular and systemic tissues was similar for male and female rats.

Following a single intravenous administration of 1 mg/kg of ¹⁴C-diquafosol to male rats (n = 4), radioactivity levels peaked at 2 (at the first timepoint) to 5 minutes post-dose in most tissues and the

radioactivity levels in the submandibular gland, kidneys, spleen, bone marrow, and submandibular lymph nodes were higher than the plasma level, and elimination of radioactivity from the tissues was slow beyond 1 hour post-dose. Whole-body autoradioluminography in rats (2 males and 2 females) showed that radioactivity was rapidly distributed throughout the body and high levels of radioactivity appeared in the bladder urine, submandibular gland, and preputial gland etc. The radioactivity distribution in ocular and systemic tissues was similar for male and female rats.

Following a single instillation of 3% ^{14}C -diquafosol into both eyes of male rabbits ($n = 2$) (50 μL in each eye, 3 mg/rabbit), the rank order of radioactivity in ocular tissues was palpebral conjunctiva > bulbar conjunctiva > cornea > sclera > extraocular muscle > aqueous humor > iris-ciliary body (radioactivity levels peaked at 30 minutes post-instillation in aqueous humor and at 5 minutes post-instillation [at the first timepoint] in other tissues) and elimination of radioactivity from the ocular tissues was slow.

3.(ii).A.(2).2 Melanin affinity (4.2.2.3-001)

The extent of binding of diquafosol (10 nmol/L) to melanin isolated from bovine eyes was $6.25 \pm 4.16\%$, indicating that diquafosol has a low affinity for melanin.

3.(ii).A.(3) Metabolism

3.(ii).A.(3).1 *In vitro* metabolites in plasma (4.2.2.4-001)

When rat, rabbit, dog, and human plasma was incubated with ^{14}C -diquafosol (10 $\mu\text{mol/L}$), the rank order of diquafosol metabolic activity was rat > rabbit > dog = human. In all animal species, UMP, uridine, and uracil were formed as metabolites and uridine was the primary metabolite in the rat and human and UMP in the rabbit and dog. The metabolic activities of rat, rabbit, and dog plasma were similar between males and females.

3.(ii).A.(3).2 *In vitro* metabolites in rabbit ocular tissues (4.2.2.4-002)

When the palpebral conjunctiva S9, bulbar conjunctiva S9, cornea S9, iris-ciliary body S9, and aqueous humor of a rabbit were incubated with ^{14}C -diquafosol (20 and 200 $\mu\text{mol/L}$), the rate of metabolism was highest in the iris-ciliary body, followed by the palpebral conjunctiva, cornea, and then bulbar conjunctiva, while little metabolism occurred in the aqueous humor. UTP, UDP, UMP, uridine, uracil, dihydrouracil, and an unknown metabolite were detected as metabolites and uridine was the primary metabolite.

3.(ii).A.(3).3 *In vitro* metabolites in human liver microsomes (4.2.2.4-003)

When human liver microsomes were incubated with ^{14}C -diquafosol (10 $\mu\text{mol/L}$), little metabolism occurred in the absence of Mg^{2+} , but diquafosol was rapidly metabolized in the presence of Mg^{2+} to form UMP, uridine, and uracil. Diquafosol was metabolized mostly to uridine (56.0%) and uracil (36.5%) after 6 hours of incubation. It was suggested that ecto alkaline phosphodiesterase I, which is known to hydrolyze dinucleotide polyphosphate and requires Mg^{2+} as a cofactor, is involved in the

metabolism of diquafosol.

3.(ii).A.(3).4) Metabolites in plasma and ocular tissues after topical ocular instillation in rabbits (4.2.2.4-004)

Following a single instillation of 3% ¹⁴C-diquafosol into both eyes of male rabbits (n = 2) (50 µL in each eye, 3 mg/rabbit), diquafosol was the predominant component in plasma at 5 minutes post-instillation, but plasma diquafosol was below the lower limit of quantification (3 times the background radioactivity) and uridine, uracil, and dihydrouracil were detected at 30 minutes post-instillation. Uridine was the predominant component in ocular tissues at 5 minutes post-instillation. At 30 minutes post-instillation, diquafosol was practically not detected and uridine, uracil, and dihydrouracil were the major components in ocular tissues. At 4 hours post-instillation, UMP was the major component in ocular tissues other than the aqueous humor and dihydrouracil in the aqueous humor.

3.(ii).A.(4) Excretion

3.(ii).A.(4).1) Excretion in urine, feces, and expired air (4.2.2.2-001, 4.2.2.2-002)

Following a single instillation of 3% ¹⁴C-diquafosol into both eyes of rats (5 males, 6 females) (5 µL in each eye, 0.3 mg/rat), the excretion rates (% of the dose) up to 168 hours post-instillation in male and female rats were 84.5 ± 3.9% and 79.4 ± 5.2%, respectively, in the expired air; 6.8 ± 1.0% and 7.6 ± 0.9%, respectively, in urine; and 4.5 ± 0.8% and 4.1 ± 0.7%, respectively, in feces. Following a single intravenous administration of 1 mg/kg of ¹⁴C-diquafosol to rats (4 males and 4 females), the excretion rates up to 168 hours post-dose in male and female rats were 84.6 ± 2.5% and 84.6 ± 3.7%, respectively, in the expired air; 11.2 ± 1.0% and 10.7 ± 0.8%, respectively, in urine; and 0.6 ± 0.1% and 0.6 ± 0.1%, respectively, in feces. It was shown that the primary route of excretion of diquafosol after topical ocular instillation or intravenous administration is in the expired air.

3.(ii).A.(4).2) Biliary excretion (4.2.2.2-001)

Following a single instillation of 3% ¹⁴C-diquafosol into both eyes of bile duct cannulated rats (4 males and 4 females) (5 µL in each eye, 0.3 mg/rat), the biliary excretion rate (% of the dose) up to 48 hours post-instillation was 0.3 ± 0.1% in male rats and 0.2 ± 0.0% in female rats and biliary excretion of diquafosol was negligible.

Based on the above results from the metabolism and excretion studies, it is postulated as follows:

Diquafosol is first hydrolyzed by ecto alkaline phosphodiesterase I to UTP and UMP. Then, like endogenous substances, UTP is metabolized through the metabolic pathway of pyrimidine nucleotides to UDP and UMP, and UMP is further metabolized to uridine, uracil, dihydrouracil, and β-ureide propionate and excreted as β-alanine in urine and as carbon dioxide in the expired air.

3.(ii).B Outline of the review by PMDA

PMDA asked the applicant to explain the association between the ocular pharmacokinetics and

therapeutic efficacy of diquafosol after topical ocular application.

The applicant explained as follows:

Although the site of action of diquafosol is at the P2Y₂ receptors localized to conjunctival epithelium and goblet cell membranes, unchanged diquafosol and UTP have comparable affinities for the P2Y₂ receptor (4.2.1.1-016) and UDP also has minimal affinity (Brunschweiler A, Müller CE. *Curr Med Chem.* 2006;13: 289-312). Thus, these three compounds should contribute to therapeutic efficacy. In a single-instillation study of 3% ¹⁴C-diquafosol in rabbits (4.2.2.4-004), unchanged diquafosol, UTP, and UDP were minor components in the palpebral and bulbar conjunctiva at 5 to 30 minutes post-instillation, and these substances are considered to be rapidly metabolized on the ocular surface and eliminated from the conjunctiva after instillation. Taking account of these findings, it is inferred that after topical ocular application of diquafosol, unchanged diquafosol and its metabolites UTP and UDP very quickly bind to the P2Y₂ receptors and exert pharmacological effects and are then metabolized rapidly to pharmacologically inactive uridine, uracil, etc.

The elimination of radioactivity from many ocular and systemic tissues was apparently slow compared with the plasma half life of radioactivity in single-dose tissue distribution studies and repeat-dose tissue distribution has not been studied. PMDA asked the applicant to predict the accumulation of diquafosol in ocular and systemic tissues after repeated administration and explain its effects on safety.

The applicant explained as follows:

Tissue radioactivity levels following 14-day topical ocular application of diquafosol 6 times daily at 1.5-hour intervals were simulated by superposition of the data from a single-instillation ocular tissue distribution study of 3% ¹⁴C-diquafosol in rabbits and a single-instillation systemic/ocular tissue distribution study of 3% ¹⁴C-diquafosol in rats. It was predicted that the radioactivity levels in rabbit plasma and cornea would increase about 1.5-fold and 3.0-fold, respectively, after repeated administration, but would reach a steady state by Day 5 and Day 14, respectively. Likewise, it was predicted that the radioactivity levels in rat plasma, bulbar conjunctiva, cornea, and duodenum would increase about 1.4-fold, 2.4-fold, 1.3-fold, and 1.2-fold, respectively, but would all reach a steady-state by Day 7. In rabbit studies with repeated instillation of 5% diquafosol 7 times daily for 6 weeks and 9 months (4.2.3.2-001, 4.2.3.2-002), there were no toxicologically relevant findings in ocular and systemic tissues. Furthermore, also in a long-term treatment study in humans, the severity or incidence of adverse drug reactions did not increase with prolonged treatment up to 52 weeks. Therefore, it is considered that tissue metabolite concentrations after repeated ocular instillation of diquafosol are not at levels affecting safety.

In a single-instillation study in rabbits (4.2.2.4-004), unchanged diquafosol was practically not detected and uridine and uracil were the major components in ocular tissues after instillation. Thus, it is considered that residual tissue radioactivity was attributable to these metabolites. Uridine is known to be taken up into cells by nucleoside transporters, which is considered associated partly with slow

elimination of radioactivity from tissues.

PMDA accepted the above explanation and concluded that although diquafosol or its metabolites are expected to be accumulated after repeated administration, accumulation is unlikely to cause any safety problem.

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

The results from the following toxicity studies of diquafosol were submitted: single-dose toxicity studies (an ocular irritation study, single intravenous dose toxicity studies); repeat-dose toxicity studies (repeated-instillation studies, repeated intravenous administration studies); genotoxicity studies; reproductive and developmental toxicity studies; and a skin sensitization study.

3.(iii).A.(1) Single-dose toxicity (4.2.3.1-001 to 4.2.3.1-003)

In an ocular irritation study, 0% (control), 1%, 3%, 8%, or 16% of diquafosol was instilled into one eye of rabbits 10 times a day (at 30-minute intervals). Diquafosol caused mild (at concentrations of 3% and 8%) or moderate (at a concentration of 16%) ocular irritation (conjunctival redness and edema) and no effects on general signs were observed.

In single intravenous dose toxicity studies, deaths, hypoactivity, irregular respiration, etc. occurred in rats; and vomiting, sedation, etc. occurred in dogs. The approximate lethal dose was determined to be 185 mg/kg in rats and >370 mg/kg in dogs.

3.(iii).A.(2) Repeat-dose toxicity

In repeated-instillation studies in rabbits (6 weeks, 9 months), diquafosol caused ocular irritation (conjunctival redness and edema). In repeated intravenous dose toxicity studies in rats (1 month, 6 months) and dogs (1 month, 9 months), the major toxicological findings were mucosal calcification and epithelial hyperplasia of the glandular stomach and calcification of the renal medulla in rats and glomerular calcification in dogs.

3.(iii).A.(2).1) Toxicity study by 6-week repeated instillation in rabbits (4.2.3.2-001)

Rabbits (5 rabbits/sex/group) received 50 µL of 0% (control), 1%, 3%, 5%, or 8% diquafosol in both eyes 7 times daily (at 2-hour intervals) for 6 weeks. Diquafosol at concentrations of ≥5% caused mild ocular irritation (conjunctival redness and edema) without associated histopathological changes, which tended to resolve after a 1-week recovery period. Thus, these findings were considered of no toxicological significance. The no observed adverse effect level (NOAEL) was determined to be 8%.

3.(iii).A.(2).2) Toxicity study by 9-month repeated instillation in rabbits (4.2.3.2-002)

Rabbits (6 rabbits/sex/group) received 50 µL of 0% (control), 1%, 3%, 5%, or 8% diquafosol in both eyes 7 times daily (at 2-hour intervals) for 9 months. Diquafosol at concentrations of ≥3% caused

ocular irritation (conjunctival redness and edema) without associated histopathological changes, but the reversibility of conjunctival redness observed at a concentration of 8% was not confirmed by examination at Month 9. Thus, the NOAEL was determined to be 5%.

3.(iii).A.(2).3 One-month repeated intravenous dose toxicity study in rats (4.2.3.2-003)

Rats (10 rats/sex/group) were intravenously administered 0 (control), 10, 30, or 100 mg/kg/day of diquafosol for 1 month. Transient auricular/limb redness at all dose levels and changes in clinical observations such as abnormal gait, abnormal respiration, hunched position, and lateral position at 100 mg/kg/day were observed. At 100 mg/kg/day, mucosal calcification and epithelial hyperplasia of the glandular stomach and calcification of the renal medulla were observed, which tended to resolve after a 1-month recovery period. The NOAEL was determined to be 30 mg/kg/day.

3.(iii).A.(2).4 Six-month repeated intravenous dose toxicity study in rats (4.2.3.2-004)

Rats (12 rats/sex/group) were intravenously administered 0 (control), 3, 10, 30, or 100 mg/kg/day of diquafosol for 6 months. Transient auricular/limb redness at all dose levels, hemosiderosis of the mesenteric lymph nodes at ≥ 10 mg/kg/day, reduced body weight gain and thickening of the pulmonary arterial media, etc. at ≥ 30 mg/kg/day, and increases in adrenal gland and spleen weights, an increase in mast cells in the mesenteric lymph nodes, and calcification of the glandular stomach, follicular cysts, and renal medulla, etc. at 100 mg/kg/day were observed. The NOAEL was determined to be 3 mg/kg/day.

3.(iii).A.(2).5 One-month repeated intravenous dose toxicity study in dogs (4.2.3.2-005)

Dogs (3 dogs/sex/group) were intravenously administered 0 (control), 50, 100, or 150 mg/kg/day of diquafosol for 1 month. There were no toxicologically significant changes and the NOAEL was determined to be 150 mg/kg/day.

3.(iii).A.(2).6 Nine-month repeated intravenous dose toxicity study in dogs (4.2.3.2-006)

Dogs (4 dogs/sex/group) were intravenously administered 0 (control), 15, 50, or 150 mg/kg/day of diquafosol for 9 months. Transient tremor at 150 mg/kg/day and glomerular calcification in females at ≥ 50 mg/kg/day and males and females at 150 mg/kg/day were observed. The NOAEL was determined to be 50 mg/kg/day for males and 15 mg/kg/day for females.

3.(iii).A.(3) Genotoxicity (4.2.3.3-001 to 4.2.3.3-004)

As genotoxicity studies, a bacterial reverse mutation assay, a chromosomal aberration assay in Chinese hamster ovary cells, a mouse lymphoma TK assay, and a mouse micronucleus assay were performed. As a result, diquafosol was determined to be non-genotoxic.

3.(iii).A.(4) Carcinogenicity

No carcinogenicity study has been performed in accordance with “Guidelines for Carcinogenicity Studies of Drugs” (PMSB/ELD Notification No. 1607 dated November 1, 1999) because of the

following facts: (a) diquafosol is non-genotoxic; (b) diquafosol is rapidly metabolized to endogenous metabolites UTP, UDP, UMP, uridine, and uracil after administration; (c) the systemic exposure of diquafosol was below the lower limit of quantification and the systemic exposure of metabolites also did not exceed their physiological concentrations in repeated-instillation studies; and (d) there was no evidence of preneoplastic lesions in repeated-instillation and repeated intravenous administration studies.

3.(iii).A.(5) Reproductive and developmental toxicity

A study of fertility and early embryonic development to implantation, embryo-fetal development studies, and a study for effects on pre- and postnatal development, including maternal function were conducted by the intravenous route. The weight of epididymis and spermatogenesis were reduced in parent animals in the study of fertility and early embryonic development to implantation and there was no evidence of teratogenicity in the embryo-fetal development studies.

3.(iii).A.(5).1) Study of fertility and early embryonic development to implantation (4.2.3.5-001)

Male and female rats (22 rats/sex/group) were intravenously administered 0 (control), 10, 30, or 100 mg/kg/day of diquafosol. Males were dosed from 29 days prior to mating until the day before necropsy and females were dosed from 15 days prior to mating until gestation day 7. Transient auricular/limb redness at all dose levels and transient hypotonia, reduced body weight gain, and reduced food consumption at 100 mg/kg/day were observed. At 100 mg/kg/day, the weight of epididymis, cauda epididymal sperm count, and the percentage of normal sperm were reduced, but there were no treatment-related effects on copulation index or fertility index, etc. The NOAELs were determined to be 30 mg/kg/day for general toxicity of parent animals, 30 mg/kg/day for paternal, reproductive and developmental toxicity, 100 mg/kg/day for maternal, reproductive and developmental toxicity, and 100 mg/kg/day for early embryonic developmental toxicity.

3.(iii).A.(5).2) Rat embryo-fetal development study (4.2.3.5-002, 4.2.3.5-003)

Pregnant rats (n = 22/group) were intravenously administered 0 (control), 30, 100, or 300 mg/kg/day of diquafosol from gestation day 6 to gestation day 17. In dams, transient auricular/limb redness at all dose levels, hypoactivity at ≥ 100 mg/kg/day, and reductions in body weight and food consumption, abnormal respiration, hypotonia, pale eyeball, convulsion, etc. at 300 mg/kg/day were observed and four deaths occurred. In fetuses, external, visceral, and skeletal examinations revealed no abnormalities and there were no findings suggestive of teratogenicity. The NOAELs were determined to be 30 mg/kg/day for maternal general toxicity and 300 mg/kg/day for maternal reproductive toxicity and embryo-fetal toxicity.

3.(iii).A.(5).3) Rabbit embryo-fetal development study (4.2.3.5-004)

Pregnant rabbits (n = 22/group) were intravenously administered 0 (control), 3, 10, or 40 (decreased to 30) mg/kg/day (since 2 rabbits were killed in extremis, the dose was reduced from gestation day 10, 12, or 13 in 9 rabbits and diquafosol treatment was started at a dose of 30 mg/kg/day in 11 rabbits) of

diquafosol from gestation day 6 to gestation day 19. Two dams in the 40 mg/kg/day group were killed in extremis due to poor general condition and convulsion, transient changes in the pupil size (9 of 22 dams had miosis, 5 of 22 dams had mydriasis), and irregular respiration etc. were observed in dams in the 40 (decreased to 30) mg/kg/day group. In fetuses, external, visceral, and skeletal examinations revealed no abnormalities and there were no findings suggestive of teratogenicity. The NOAELs were determined to be 10 mg/kg/day for maternal general toxicity and 30 mg/kg/day for maternal reproductive toxicity and embryo-fetal toxicity.

3.(iii).A.(5).4) Rat study for effects on pre- and postnatal development, including maternal function (4.2.3.5-005)

Pregnant rats (n = 21-22/group) were intravenously administered 0 (control), 10, 30, or 100 mg/kg/day of diquafosol from gestation day 6 to lactation day 20. In dams, transient auricular/limb redness at all dose levels and reduced body weight gain at 100 mg/kg/day were observed. There were no treatment-related effects on the gestation length, parturition, or lactation of dams or the clinical observations, reproductive function, or behavior of the offspring. The NOAELs were determined to be 30 mg/kg/day for maternal general toxicity and 100 mg/kg/day for maternal reproductive toxicity and the offspring.

3.(iii).A.(6) Other toxicity study

Skin sensitization study in guinea pigs (4.2.3.7-001)

The skin sensitization potential of 0% (control), 1%, 3%, and 8% diquafosol was assessed in guinea pigs (6 females/group) by the adjuvant and patch test method. No skin reaction was observed in any animal and it was concluded that diquafosol is not a skin sensitizer.

3.(iii).B Outline of the review by PMDA

Concerning changes in the pupil size observed in dams in a rabbit embryo-fetal development study, PMDA asked the applicant to discuss its mechanism of development and relevance for human safety.

The applicant explained as follows:

Although it has been reported that P2Y receptors may regulate the pupil size either by controlling the contraction/relaxation of the pupillary sphincter and dilator muscles or by modulating the sympathetic nervous system (Pintor J et al. *Purinergic Signal*. 2004;1: 83-90), the association between diquafosol and changes in the pupil size is unclear. Although changes in the pupil size occurred, with serious clinical signs such as irregular respiration after intravenous administration of high-dose diquafosol, diquafosol at concentrations higher than the clinical concentration had no effect on the pupil size in ocular irritation and repeated-instillation studies in rabbits and phase I clinical studies. Therefore, it is unlikely that topical ocular application of diquafosol affect the pupil size.

Concerning auricular/limb redness observed in rat intravenous administration studies and conjunctival redness observed in rabbit repeat-dose studies, PMDA asked the applicant to discuss their relationship

to diquafosol and relevance for human safety.

The applicant explained as follows:

As it has been reported that P2Y₂ receptor agonists act on vascular endothelial cells to release prostaglandin I₂ and carbon monoxide resulting in vasodilatation (Erlinge D, Burnstock G. *Purigeric Signal*. 2008;4: 1-20), it cannot be ruled out that auricular/limb redness observed in the rat repeated intravenous dose toxicity studies and conjunctival redness observed in the rabbit repeat-dose studies were both induced by diquafosol or its metabolites acting on vascular endothelial cells. On the other hand, also in clinical studies, as an adverse event possibly relevant to these findings, conjunctival hyperaemia has been reported. However, as the incidence of conjunctival hyperaemia among the short-term treatment population was 2.8% (4 of 141 subjects) in the placebo group, 4.2% (4 of 96 subjects) in the 1% diquafosol group, and 2.4% (7 of 290 subjects) in the 3% diquafosol group and there were no differences between the placebo and diquafosol groups, conjunctival hyperaemia is unlikely to be related to topical ocular application of diquafosol.

PMDA largely accepted the above responses and considers as follows:

There is no major problem with the toxicity of diquafosol after topical ocular application. However, taking into account that the NOAEL in a toxicity study by 9-month repeated instillation in rabbits (5%) is close to the clinical concentration (3%), the possibility of the development of conjunctival hyperaemia associated with pharmacological effects of diquafosol also in clinical use can not be ruled out. Therefore, it is necessary to carefully evaluate clinical safety data from clinical studies and post-marketing surveillance, etc.

4. Clinical data

4.(i) Summary of biopharmaceutics and clinical pharmacology studies

4.(i).A Summary of the submitted data

As the evaluation data on the pharmacokinetics of diquafosol, the results from a phase I study with a single instillation/frequent instillation per day (00890003 [5.3.3.1-001]) and a phase I repeated-instillation study (00890103 [5.3.3.1-003]) in Japanese healthy adult male subjects were submitted. Plasma concentrations of diquafosol and its metabolites UTP, UDP, and UMP were determined using LC-MS/MS, and plasma uridine concentrations were determined using HPLC-UV (Lower limit of quantification, 2 ng/mL for diquafosol, 20 ng/mL for UTP and UDP, 50 ng/mL for UMP, 500 ng/mL for uridine). Unless otherwise specified, pharmacokinetic parameters are expressed as the mean \pm SD. Studies using human biomaterials are described as part of non-clinical pharmacokinetic studies.

4.(i).A.(1) Studies in healthy adult subjects

4.(i).A.(1).1 Phase I study with a single instillation/frequent instillation per day (5.3.3.1-001, 00890003 [20 to 20])

Following single escalating doses of 0.3%, 1%, 3%, and 5% diquafosol ophthalmic solution instilled into both eyes of Japanese healthy adult male subjects (n = 8, 20-26 years) (the doses were separated by 6-day washout periods), the pharmacokinetics of diquafosol and its metabolites (UTP, UDP, UMP) were determined. Plasma concentrations of diquafosol, UTP, UDP, and UMP up to 1 hour after instillation were as shown in Table 1.

Table 1. Plasma concentrations of diquafosol and its metabolites over time following a single instillation of diquafosol ophthalmic solution in to the eyes of Japanese healthy adult male subjects (ng/mL)

Analyte	Diquafosol concentration	Before instillation	5 minutes after instillation	15 minutes after instillation	30 minutes after instillation	1 hour after instillation
Diquafosol	0.3%, 1%, 3%, 5%	BLQ	BLQ	BLQ	BLQ	BLQ
UTP	0.3%	69.96 ± 22.82	142.35 ± 39.76	179.84 ± 53.98	163.29 ± 47.94	188.79 ± 51.54
	1%	193.82 ± 61.05	192.50 ± 57.69	187.44 ± 50.73	200.27 ± 59.33	183.24 ± 57.02
	3%	159.46 ± 33.36	175.43 ± 26.91	225.45 ± 51.13	200.28 ± 28.83	222.48 ± 48.75
	5%	119.33 ± 34.30	120.21 ± 32.52	128.45 ± 26.03	123.76 ± 27.12	137.05 ± 40.85
UDP	0.3%	23.42 ± 16.48	52.37 ± 24.15	48.63 ± 15.16	57.54 ± 21.97	63.07 ± 23.62
	1%	63.22 ± 11.81	59.75 ± 14.06	57.69 ± 15.84	66.09 ± 17.04	56.74 ± 14.19
	3%	79.26 ± 13.81	89.40 ± 16.94	98.36 ± 18.67	101.43 ± 16.01	96.25 ± 21.67
	5%	55.64 ± 15.69	61.12 ± 15.46	56.76 ± 12.83	62.70 ± 13.35	63.67 ± 20.52
UMP	0.3%, 1%, 3%, 5%	BLQ	BLQ	BLQ	BLQ	BLQ

BLQ: below the lower limit of quantification

Mean ± SD

Japanese healthy adult male subjects (n = 8, 20-23 years) received escalating doses of 3% and 5% diquafosol ophthalmic solution separated by a 6-day washout period. Following instillation of one drop of diquafosol solution into both eyes 6 times a day at 2-hour intervals, the pharmacokinetics of diquafosol and its metabolites (UTP, UDP, UMP) were determined. Plasma concentrations of diquafosol, UTP, UDP, and UMP up to 1 hour after the first instillation and the sixth instillation were as shown in Table 2.

Table 2. Plasma concentrations of diquafosol and its metabolites over time following instillation of diquafosol ophthalmic solution 6 times a day in Japanese healthy adult male subjects (ng/mL)

First instillation						
Analyte	Diquafosol concentration	Before instillation	5 minutes after instillation	15 minutes after instillation	30 minutes after instillation	1 hour after instillation
Diquafosol	3%, 5%	BLQ	BLQ	BLQ	BLQ	BLQ
UTP	3%	215.75 ± 42.78	161.97 ± 45.55	169.78 ± 51.63	164.62 ± 41.96	164.34 ± 47.66
	5%	234.54 ± 106.25	191.69 ± 62.98	205.65 ± 73.44	222.74 ± 59.39	202.72 ± 38.73
UDP	3%	68.74 ± 31.60	55.45 ± 26.50	55.95 ± 30.31	51.59 ± 19.17	51.68 ± 26.55
	5%	86.16 ± 34.96	72.12 ± 21.81	75.57 ± 27.11	70.57 ± 22.84	74.71 ± 19.05
UMP	3%, 5%	BLQ	BLQ	BLQ	BLQ	BLQ
Sixth instillation						
Diquafosol	3%, 5%	—	BLQ	BLQ	BLQ	BLQ
UTP	3%	—	154.04 ± 34.28	168.51 ± 35.11	170.13 ± 52.40	167.60 ± 32.84
	5%	—	177.64 ± 50.20	207.39 ± 39.59	191.86 ± 40.80	180.00 ± 26.64
UDP	3%	—	44.24 ± 27.92	46.22 ± 18.30	50.43 ± 37.22	55.15 ± 27.71
	5%	—	63.99 ± 20.68	63.81 ± 17.88	69.83 ± 17.66	66.77 ± 25.67
UMP	3%, 5%	—	BLQ	BLQ	BLQ	BLQ

BLQ: below the lower limit of quantification, —: Not measured

Mean ± SD

Following both single and frequent instillation, diquafosol was below the lower limit of quantification (2 ng/mL) at all sampling points. UMP was below the lower limit of quantification (50 ng/mL) at all sampling points. Although UTP and UDP were quantifiable at all sampling points following both single and frequent instillation, UTP and UDP concentrations remained almost constant regardless of the dose of diquafosol and the time after instillation, and similar levels of UTP and UDP were detected even before instillation. Thus, it was inferred that the majority of UTP and UDP were those produced by endogenous processes.

4.(i).A.(1).2) Phase I repeated-instillation study (5.3.3.1-003, 00890103 [■ 20■ to ■ 20■])

Japanese healthy adult male subjects (24 subjects, 20-27 years) received one drop of 3% or 5% diquafosol ophthalmic solution or placebo in both eyes 6 times daily for 7 consecutive days. The pharmacokinetics of diquafosol and its metabolites (UTP, UDP, UMP, uridine) were determined.

Plasma diquafosol concentrations before instillation and at 5, 15, and 30 minutes and 1 hour after instillation on Day 1 and Day 7 were all below the lower limit of quantification (2 ng/mL) in all groups. Plasma UTP was quantifiable at all sampling points in all groups and remained almost constant regardless of the dose of diquafosol and the time after instillation, and the time course of plasma UTP concentrations was similar for all 3 groups on both Days 1 and 7. Plasma uridine was quantifiable at all sampling points in all groups and tended to decrease after instillation compared with before instillation in all groups. Plasma uridine concentrations were higher in the 3% diquafosol group compared with the 5% diquafosol group and the placebo group on Day 1, while plasma uridine concentrations were similar for all 3 groups on Day 7. Plasma UMP was below the lower limit of quantification (50 ng/mL) at all sampling points in all groups. As plasma UTP concentrations may have been overestimated due to suppression of the peak area of the internal standard in the samples, they are treated as reference values only. Because plasma UDP concentrations could not be calculated due to poor peak shape, they are reported as no data.

4.(i).B Outline of the review by PMDA

Since dry eye patients have corneal/conjunctival damage, PMDA asked the applicant to explain whether differences in the pharmacokinetics of diquafosol according to the severity of the disease may affect safety.

The applicant explained as follows:

Because corneal/conjunctival damage is more extensive in patients with severe dry eye, the amount of diquafosol distributed into ocular tissues may be increased, resulting in higher diquafosol and metabolite concentrations in ocular tissues. However, when the incidence of local ocular adverse events (with an incidence $\geq 1\%$) was analyzed by severity of dry eye based on fluorescein staining (FL) score (scores of 1-3, 4-6, and 7-9) using the data from subjects treated with 3% diquafosol ophthalmic solution in early phase II, late phase II, and phase III studies, there was no FL score-dependent increase in the number of adverse events. Therefore, even if ocular distribution of diquafosol is altered

according to the severity of dry eye, safety should be unaffected.

PMDA accepts the above explanation at present, but considers that due to the limited number of patients with severe dry eye assigned to the 3% diquafosol group in Japanese clinical studies (especially, only 39 patients had a score of ≥ 7), it is necessary to further assess safety by dry eye severity via post-marketing surveillance.

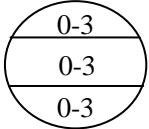
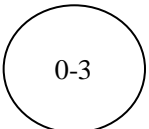
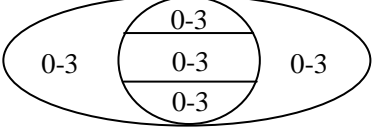
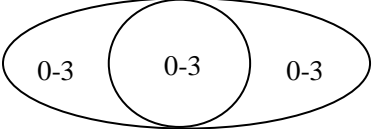
4.(ii) Summary of clinical efficacy and safety

4.(ii).A Summary of the submitted data

As the efficacy and safety evaluation data, the results from an early phase II study (00890207 [5.3.5.1-001]), a late phase II study (00890404 [5.3.5.1-002]), a phase III study (00890602 [5.3.5.1-003]), a long-term study (1) (00890405 [5.3.5.2-001]), and a long-term study (2) (00890603 [5.3.5.2-003]) involving dry eye patients were submitted. As the safety evaluation data, the results from a phase I study with a single instillation/frequent instillations per day (00890003 [5.3.3.1-001]) and a phase I repeated-instillation study (00890103 [5.3.3.1-003]) involving healthy adult subjects were submitted.

In clinical studies in dry eye patients, FL and Rose Bengal staining (RB) were graded according to a modified version of the scoring system of the Definition and Diagnostics of Dry Eye 1995, as shown in Figure 1.

Figure 1. Comparison of the scoring system used in clinical studies with that of the Definition and Diagnostics of Dry Eye

Clinical studies of diquafosol	Definition and Diagnostics of Dry Eye 1995
FL 	FL 
RB 	RB 

Staining of each region is graded on a 0-3 scale and the scores of all regions are summed.

4.(ii).A.(1) Studies in healthy adult subjects

4.(ii).A.(1).1 Phase I study with a single instillation/frequent instillations per day (5.3.3.1-001, 00890003 [■ 20■ to ■ 20■])

An open-label, uncontrolled study was conducted to evaluate the safety and pharmacokinetics of a single instillation and 6 instillations of diquafosol ophthalmic solution per day in Japanese healthy adult male subjects (Target sample size of 16 [8 subjects for each step]) [for pharmacokinetics, see 4.(i) Summary of biopharmaceutics and clinical pharmacology studies].

In a study with a single instillation, one drop of 0.3% (Step 1), 1% (Step 2), 3% (Step 3), or 5% (Step 4) diquafosol ophthalmic solution was to be instilled into both eyes once a day. In a study with 6 instillations per day, one drop of 3% (Step 5) or 5% (Step 6) diquafosol ophthalmic solution was to be instilled into both eyes 6 times a day. Steps 1 to 4 and Steps 5 to 6 were conducted respectively, using a within-subject design, and the steps were separated by 6-day washout periods.

A total of 16 subjects were treated, of whom 8 subjects received 4 different doses of Step 1 to Step 4, and 8 subjects received 2 different doses of Step 5 and Step 6. The information on a total of 48 cases was collected and all subjects were included in the safety analysis.

No adverse events (excluding abnormal laboratory changes) were reported. Abnormal laboratory changes reported were neutrophils increased in 1 subject treated with 1% diquafosol and urobilinogen urine positive in 1 subject treated with 3% diquafosol in a single instillation study and AST (GOT) increased/ALT (GPT) increased/ γ -GTP increased in 1 subject treated with 5% diquafosol in a study with 6 instillations per day. The events were all transient and returned to the reference ranges without treatment and their causal relationship to study drug was denied.

Based on the above, the applicant explained that there should be no major problem with the safety and tolerability of a single instillation of 0.3% to 5% diquafosol and 6 instillations of 3% or 5% diquafosol per day.

4.(ii).A.(1).2) Phase I repeated-instillation study (5.3.3.1-003, 00890103 [■■ 20■■ to ■■ 20■■])

A placebo-controlled, double-blind study was conducted to evaluate the safety and pharmacokinetics of repeated-instillation of diquafosol ophthalmic solution in Japanese healthy adult male subjects (Target sample size of 24 [8 subjects in the diquafosol group and 4 subjects in the placebo group for each step]) [for pharmacokinetics, see 4.(i) Summary of biopharmaceutics and clinical pharmacology studies].

One drop of 3% (Step 7) or 5% (Step 8) diquafosol ophthalmic solution or placebo was to be instilled into both eyes 6 times daily for 7 days.

All of 24 treated subjects (8 subjects in the diquafosol group and 4 subjects in the placebo group for each step) were included in the safety analysis.

No adverse events (excluding abnormal laboratory changes) were reported. Abnormal laboratory changes reported were monocytes increased in 1 subject treated with 3% diquafosol, urinary sediment/white blood cell increased in 1 subject treated with 5% diquafosol and white blood cell count increased in 1 subject treated with 5% diquafosol, and monocytes increased in 1 subject treated with placebo. The events were all transient and returned to the reference ranges without treatment and their causal relationship to study drug was denied.

Based on the above, the applicant explained that there should be no major problem with the safety and tolerability of repeated-instillation of 3% or 5% diquafosol (6 times daily for 7 days).

4.(ii).A.(2) Studies in dry eye patients

4.(ii).A.(2).1 Early phase II study (5.3.5.1-001, 00890207 [■■ 20■■ to ■■ 20■■])

A placebo-controlled, randomized, double-blind, parallel-group, comparative study was conducted to evaluate the efficacy and safety of diquafosol ophthalmic solution in dry eye patients² (Target sample size of 80 [40 subjects per group]).

One drop of 3% diquafosol ophthalmic solution or placebo was to be instilled into the eyes of patients diagnosed as having definite dry eye, 6 times daily for 6 weeks.

All of 97 treated subjects (50 subjects in the diquafosol group, 47 subjects in the placebo group) were included in the safety analysis, of whom 95 subjects (48 subjects in the diquafosol group, 47 subjects in the placebo group) were included in the Full Analysis Set (FAS) and the primary efficacy analysis was based on the FAS. Excluded were 1 subject who received a prohibited concomitant medication and 1 subject who received prohibited concomitant therapy.

The difference from placebo in the change from baseline in the FL score (the primary efficacy endpoint) (diquafosol vs. placebo; point estimate [95% confidence interval (CI)]) was -0.19 [-1.03, 0.65] at Week 2, -0.86 [-1.62, -0.09] at Week 4, and -0.51 [-1.33, 0.30] at Week 6 or discontinuation, and the between-treatment difference in the change from baseline in the RB score (point estimate [95% CI]) was -0.70 [-1.47, 0.07] at Week 2, -0.80 [-1.68, 0.07] at Week 4, and -0.57 [-1.38, 0.24] at Week 6 or discontinuation.

Adverse events (excluding abnormal laboratory changes) occurred in 38.0% of the diquafosol group (19 of 50 subjects) (35 events) and 29.8% of the placebo group (14 of 47 subjects) (18 events). There were no fatal or serious adverse events. Adverse events leading to treatment discontinuation were reported by 1 subject in the diquafosol group and 1 subject in the placebo group and a causal relationship to study drug could not be denied for the event in the placebo group (corneal epithelium defect), but the event resolved following study drug discontinuation.

Adverse drug reactions (excluding abnormal laboratory changes) occurred in 22.0% of the diquafosol group (11 of 50 subjects) (14 events) and 19.1% of the placebo group (9 of 47 subjects) (10 events). The events reported by at least 3% of subjects in either group were headache (4.3% [2 of 47 subjects] in the placebo group), abnormal sensation in eye (4.0% [2 of 50 subjects] in the diquafosol group,

² Patients who were diagnosed with dry eye based on the Definition and Diagnostics of Dry Eye¹⁹⁹⁵ ([a] Schirmer's I test ≤ 5 mm in 5 minutes or tear film break-up time ≤ 5 seconds and [b] FL score ≥ 1 [for a maximum score of 3] or RB score ≥ 3 [for a maximum score of 9]) and had a FL score of ≥ 1 according to the scoring system for diquafosol ophthalmic solution at the end of a run-in period.

2.1% [1 of 47 subjects] in the placebo group), eye discharge (6.0% [3 of 50 subjects] in the diquafosol group), eye irritation (14.0% [7 of 50 subjects] in the diquafosol group, 2.1% [1 of 47 subjects] in the placebo group), and eye pain (4.3% [2 of 47 subjects] in the placebo group).

Abnormal laboratory changes occurred in 4.0% of the diquafosol group (2 of 50 subjects) (2 events) and 2.1% of the placebo group (1 of 47 subjects) (1 event). The 1 event in the diquafosol group (BUN increased) was classified as an adverse drug reaction.

Based on the above, the applicant explained that as the changes in the FL and RB scores in the diquafosol group were greater than those in the placebo group at all timepoints, the efficacy of diquafosol in dry eye treatment was suggested and there were no clinically relevant safety problems.

4.(ii).A.(2).2) Late phase II study (5.3.5.1-002, 00890404 [■ 20■ to ■ 20■])

A placebo-controlled, randomized, double-blind, parallel-group, comparative study was conducted to investigate the dose response of the efficacy and safety of diquafosol ophthalmic solution in dry eye patients³ (Target sample size of 270 [90 subjects per group]).

One drop of 1% or 3% diquafosol ophthalmic solution or placebo was to be instilled into the eyes of patients diagnosed as having definite dry eye 6 times daily for 6 weeks.

All of 286 treated subjects (96 subjects in the 1% diquafosol group, 96 subjects in the 3% diquafosol group, 94 subjects in the placebo group) were included in the safety analysis, of whom 283 subjects (95 subjects in the 1% diquafosol group, 95 subjects in the 3% diquafosol group, 93 subjects in the placebo group) were included in the FAS. Excluded were 3 subjects without available efficacy data. After further excluding 4 subjects (1 subject who received a prohibited concomitant medication, 2 subjects treated for an inadequate duration, and 1 subject with poor compliance regarding dosing frequency), 279 subjects (93 subjects in the 1% diquafosol group, 93 subjects in the 3% diquafosol group, 93 subjects in the placebo group) were included in the Per Protocol Set (PPS) and the primary efficacy analysis was based on the PPS.

The primary efficacy endpoint of the mean change from baseline in the FL score at Week 4 or early discontinuation was as shown in Table 3. In the primary analysis, both the linear contrast of dose-response relationships among the placebo, 1% diquafosol, and 3% diquafosol groups (-1, 0, 1), and the contrast of dose-response relationship, saturated at $\geq 1\%$ (-2, 1, 1), were significant. As the secondary analysis, comparison between the placebo and diquafosol groups was performed and the 3% diquafosol group exhibited a significantly greater reduction in the score compared with the placebo group, as shown in Table 3.

³ Patients who were diagnosed with dry eye based on the Definition and Diagnostics of Dry Eye 1995 and had a FL score of ≥ 1 according to the scoring system for diquafosol ophthalmic solution at the end of a run-in period.

Table 3. Dose response for mean change in FL score at Week 4 or early discontinuation

Treatment group	N	Change (Mean \pm SD)	Test results
Placebo	93	-0.95 \pm 1.33	Contrast tests for dose response relationship* (placebo, 1% diquafosol, 3% diquafosol) [-1, 0, 1], $P = 0.004$; [-2, 1, 1], $P = 0.006$ Between treatment comparison of diquafosol vs. placebo (t-test, a closed testing procedure starting with 3% diquafosol vs. placebo) 1% diquafosol, $P = 0.051$; 3% diquafosol, $P = 0.002$
1% diquafosol	93	-1.34 \pm 1.43	
3% diquafosol	93	-1.55 \pm 1.30	

*: Permutation-adjusted P -values for the maximum contrast method

Adverse events (excluding abnormal laboratory changes) occurred in 32.3% of the 1% diquafosol group (31 of 96 subjects) (49 events), 46.9% of the 3% diquafosol group (45 of 96 subjects) (79 events), and 35.1% of the placebo group (33 of 94 subjects) (65 events). No fatal adverse events were reported. As a serious adverse event, lung adenocarcinoma was reported by 1 subject in the 3% diquafosol group, but its causal relationship to study drug was denied. Adverse events leading to treatment discontinuation were reported by 4 subjects in the 1% diquafosol group, 5 subjects in the 3% diquafosol group, and 1 subject in the placebo group and a causal relationship to study drug could not be denied for the events reported by 2 subjects in the 1% diquafosol group (eye irritation and eyelid oedema; eye pain), 3 subjects in the 3% diquafosol group (eye irritation, foreign body sensation in eyes, photophobia, eye pain, and abnormal sensation in eye; eye pain; conjunctival hyperaemia), and 1 subject in the placebo group (dizziness, nasal congestion, and eye pain), but all of these events resolved following study drug discontinuation.

Adverse drug reactions (excluding abnormal laboratory changes) occurred in 12.5% of the 1% diquafosol group (12 of 96 subjects) (16 events), 15.6% of the 3% diquafosol group (15 of 96 subjects) (23 events), and 13.8% of the placebo group (13 of 94 subjects) (28 events). The events reported by at least 3% of subjects in any group were eye discharge (2.1% [2 of 96 subjects] in the 1% diquafosol group, 3.2% [3 of 94 subjects] in the placebo group), eye irritation (7.3% [7 of 96 subjects] in the 1% diquafosol group, 12.5% [12 of 96 subjects] in the 3% diquafosol group, 3.2% [3 of 94 subjects] in the placebo group), eye pain (1.0% [1 of 96 subjects] in the 1% diquafosol group, 4.2% [4 of 96 subjects] in the 3% diquafosol group, 3.2% [3 of 94 subjects] in the placebo group), foreign body sensation in eyes (1.0% [1 of 96 subjects] in the 3% diquafosol group, 3.2% [3 of 94 subjects] in the placebo group), and conjunctival hyperaemia (3.1% [3 of 96 subjects] in the 1% diquafosol group, 1.0% [1 of 96 subjects] in the 3% diquafosol group, 3.2% [3 of 94 subjects] in the placebo group).

Abnormal laboratory changes occurred in 8.3% of the 3% diquafosol group (8 of 96 subjects) (12 events) and 4.3% of the placebo group (4 of 94 subjects) (4 events). Five of the 12 events in the 3% diquafosol group (AST [GOT] increased [2], ALT [GPT] increased [2], Al-P increased [1]) were classified as adverse drug reactions.

Based on the above, the applicant explained that as 3% diquafosol showed higher efficacy than 1% diquafosol and there were also no clinically relevant safety problems, the optimum concentration of diquafosol ophthalmic solution should be 3%.

4.(ii).A.(2).3 Long-term study (1) (5.3.5.2-001, 00890405 [20 to 20])

An open-label, uncontrolled study was conducted to evaluate the safety and efficacy of long-term instillation of diquafosol ophthalmic solution in dry eye patients⁴ (Target sample size of ≥118).

One drop of 3% diquafosol ophthalmic solution was to be instilled into both eyes 6 times daily for 28 weeks.

All of 121 treated subjects were included in the safety analysis, of whom 120 subjects were included in the FAS and the primary efficacy analysis was based on the FAS. Excluded was 1 subject without available efficacy data.

The results for efficacy endpoints were as follows: the mean change from baseline in the FL score (mean ± SD) was -1.77 ± 1.32 (118 subjects) at Week 12 and -1.94 ± 1.58 (114 subjects) at Week 28 and the mean change from baseline in the RB score (mean ± SD) was -2.30 ± 2.11 (118 subjects) at Week 12 and -2.81 ± 2.43 (114 subjects) at Week 28.

Adverse events (excluding abnormal laboratory changes) occurred in 58.7% of subjects (71 of 121 subjects) (141 events). No fatal adverse events were reported. As a serious adverse event, gastritis was reported by 1 subject, but its causal relationship to study drug was denied. Adverse events leading to treatment discontinuation were reported by 3 subjects (musculoskeletal pain, eye pruritus, eye pain) and a causal relationship to study drug could not be denied for all events, but the events resolved following study drug discontinuation.

Adverse drug reactions (excluding abnormal laboratory changes) occurred in 24.0% of subjects (29 of 121 subjects) (47 events). The events reported by at least 3% of subjects were eye discharge (8.3%) (10 of 121 subjects), eye irritation (3.3%) (4 of 121 subjects), conjunctival hyperaemia (5.8%) (7 of 121 subjects), and eye pruritus (4.1%) (5 of 121 subjects).

Abnormal laboratory changes occurred in 14.0% of subjects (17 of 121 subjects) (27 events), of which 14 events (neutrophils increased [2], protein urine positive [1], urine sugar positive [1], BUN increased [1], lymphocytes decreased [1], platelet count decreased [1], ALT [GPT] increased [1], γ -GTP increased [1], bilirubin total increased [1], cholesterol total decreased [1], eosinophils increased [1], white blood cell increased [1], white blood cell decreased [1]) were classified as adverse drug reactions.

Based on the above, the applicant explained that the long-term efficacy of diquafosol ophthalmic

⁴ Patients who were diagnosed with dry eye based on the Definition and Diagnostics of Dry Eye 1995 and had a FL score of ≥1 according to the scoring system for diquafosol ophthalmic solution at the end of a run-in period.

solution for up to 28 weeks in dry eye patients was demonstrated and there were no clinically relevant safety problems.

4.(ii).A.(2).4 Phase III study (5.3.5.1-003, 00890602 [20 to 20])

A 0.1% HA ophthalmic solution-controlled, randomized, double-blind, parallel-group, comparative study was conducted to evaluate the efficacy and safety of diquafosol ophthalmic solution in dry eye patients⁵ (Target sample size of 270 [135 subjects per group]).

One drop of 3% diquafosol ophthalmic solution or 0.1% HA ophthalmic solution was to be instilled into the eyes of patients diagnosed as having definite dry eye 6 times daily for 4 weeks.

A non-inferiority margin of 0.34 for the study (between-treatment difference in the mean change in the FL score [diquafosol - HA]) was chosen to demonstrate that diquafosol preserves more than half of the effect of HA over placebo, based on the difference in the change in the FL score between the vehicle and HA groups at Week 4 of 0.68 among a subgroup of patients with a Schirmer’s I test score of ≤ 5 mm in a phase III study of 0.1% HA ophthalmic solution.

All of 287 treated subjects (144 subjects in the diquafosol group, 143 subjects in the HA group) were included in the safety analysis, of whom 286 subjects (144 subjects in the diquafosol group, 142 subjects in the HA group) were included in the FAS and the primary efficacy analysis was based on the FAS. Excluded was 1 subject without available efficacy data.

The primary efficacy endpoints were the mean changes from baseline in the FL and RB scores at the end of treatment (at Week 4 or discontinuation) and if the non-inferiority of diquafosol to HA for the change in the FL score was demonstrated, the superiority of diquafosol to HA for the change in the RB score was to be tested. The mean changes in the FL score were as shown in Table 4. As the upper limit of the two-sided 95% CI for the between-treatment difference was less than the predefined non-inferiority margin of 0.34, the non-inferiority of diquafosol to HA was demonstrated. The mean changes in the RB score were as shown in Table 5 and the superiority of diquafosol to HA was demonstrated ($P = 0.010$, t-test).

Table 4. Mean changes in FL score (FAS)

	Diquafosol	HA
N	144	142
Mean \pm SD	-2.12 \pm 1.66	-2.08 \pm 1.52
Difference from HA [95% CI]	-0.03 [-0.405, 0.338]	

Table 5. Mean changes in RB score (FAS)

	Diquafosol	HA
N	144	141
Mean \pm SD	-3.06 \pm 2.27	-2.38 \pm 2.08
Difference from HA [95% CI]	-0.67 [-1.18, -0.16]	

⁵ Patients who were diagnosed with dry eye based on the Definition and Diagnostics of Dry Eye 1995 and had a FL score of ≥ 3 and a RB score of ≥ 3 according to the scoring system for diquafosol ophthalmic solution at the end of a run-in period.

Adverse events (excluding abnormal laboratory changes) occurred in 26.4% of the diquafosol group (38 of 144 subjects) (54 events) and 18.9% of the HA group (27 of 143 subjects) (51 events). No fatal adverse events were reported. As a serious adverse event, gastrointestinal haemorrhage was reported by 1 subject in the HA group, but its causal relationship to study drug was denied. Adverse events leading to treatment discontinuation were reported by 2 subjects in the diquafosol group and 1 subject in the HA group and a causal relationship to study drug could not be denied for the events reported by 2 subjects in the diquafosol group (eye irritation; foreign body sensation in eyes and conjunctival hyperaemia), but these events resolved following study drug discontinuation.

Adverse drug reactions (excluding abnormal laboratory changes) occurred in 15.3% of the diquafosol group (22 of 144 subjects) (32 events) and 4.9% of the HA group (7 of 143 subjects) (10 events). The event reported by at least 3% of subjects in either group was eye irritation (6.3% [9 of 144 subjects] in the diquafosol group, 0.7% [1 of 143 subjects] in the HA group).

Abnormal laboratory changes occurred in 3.5% of the diquafosol group (5 of 144 subjects) (9 events) and 4.2% of the HA group (6 of 143 subjects) (19 events), of which 4 events in the diquafosol group (protein urine positive [1], white blood cell count increased [1], neutrophil count decreased [1], eosinophil count increased [1]) and 3 events in the HA group (BUN increased [1], creatinine increased [1], uric acid increased [1]) were classified as adverse drug reactions.

Based on the above, the applicant explained as follows:

The non-inferiority of diquafosol to HA for the change in the FL score and the superiority of diquafosol to HA for the change in the RB score were demonstrated, showing the efficacy of diquafosol in dry eye treatment. Although the incidence of adverse drug reactions was higher with diquafosol compared with HA, all events were mild in severity and reversible. Hence, there were no clinically relevant safety problems.

4.(ii).A.(2).5 Long-term study (2) (5.3.5.2-003, 00890603 [■ 20■ to ■ 20■])

An open-label, uncontrolled study was conducted to evaluate the safety and efficacy of long-term instillation of diquafosol ophthalmic solution in dry eye patients⁶ (Target sample size of ≥220 for 28-week treatment and ≥118 for 52-week treatment).

One drop of 3% diquafosol ophthalmic solution was to be instilled into both eyes 6 times daily for 28 or 52 weeks.

All of 244 treated subjects were included in the safety analysis, of whom 243 subjects, excluding 1

⁶ Patients who were diagnosed with dry eye based on the Definition and Diagnostics of Dry Eye 1995 and had a FL score of ≥1 according to the scoring system for diquafosol solution at the end of a run-in period.

subject without available efficacy data, were included in the FAS and the primary efficacy analysis was based on the FAS. Of 227 subjects who completed treatment up to Week 28, 119 subjects entered the extension phase and 112 subjects completed treatment up to Week 52.

The results for efficacy endpoints were as follows: the mean change from baseline in the FL score (mean \pm SD) was -1.54 ± 1.27 (236 subjects) at Week 12, -1.78 ± 1.36 (227 subjects) at Week 28, -1.80 ± 1.34 (116 subjects) at Week 40, and -1.83 ± 1.35 (112 subjects) at Week 52 and the mean change from baseline in the RB score (mean \pm SD) was -1.77 ± 2.07 (236 subjects) at Week 12, -1.93 ± 2.04 (227 subjects) at Week 28, -1.53 ± 1.79 (116 subjects) at Week 40, and -1.54 ± 1.84 (112 subjects) at Week 52.

Adverse events (excluding abnormal laboratory changes) occurred in 71.3% of subjects (174 of 244 subjects) (417 events). No fatal adverse events were reported. As serious adverse events, osteoarthritis (2 subjects), intraductal papilloma of breast (1 subject), and sudden hearing loss (1 subject) were reported, but a causal relationship to study drug was denied for all events. Adverse events leading to treatment discontinuation were reported by 4.9% of subjects (12 of 244 subjects) and a causal relationship to study drug could not be denied for the events reported by 3 subjects (photophobia; vision blurred; conjunctival hyperaemia and abnormal sensation in eye), but these events resolved or improved to a medically acceptable level on the day of study drug discontinuation or after study drug discontinuation.

Adverse drug reactions (excluding abnormal laboratory changes) occurred in 25.8% of subjects (63 of 244 subjects) (88 events). The events reported by at least 3% of subjects were eye discharge (5.7%) (14 of 244 subjects), eye irritation (4.9%) (12 of 244 subjects), eye pain (4.1%) (10 of 244 subjects), and conjunctival hyperaemia (5.3%) (13 of 244 subjects).

Abnormal laboratory changes occurred in 7.8% of subjects (19 of 244 subjects) (30 events), of which 6 events (white blood cell count increased [1], neutrophil count decreased [1], lymphocyte count increased [1], eosinophil count increased [1], BUN increased [1], K increased [1]) were classified as adverse drug reactions.

Based on the above, the applicant explained that the long-term efficacy of diquafosol ophthalmic solution for up to 52 weeks in dry eye patients was demonstrated and there were no clinically relevant safety problems.

4.(ii).B Outline of the review by PMDA

4.(ii).B.(1) Efficacy

4.(ii).B.(1).1 Justification of the scoring system used in clinical studies of diquafosol

PMDA asked the applicant to explain the basis for, and justification of, the scoring system for fluorescein and rose bengal staining used in clinical studies of diquafosol ophthalmic solution.

The applicant explained as follows:

In order to determine the condition of dry eye, not only the presence or absence of corneal epithelial damage, but also the assessment of its distribution is important (Ohashi Y, Superficial punctate keratopathy, In: Manabe R ed. *Cornea*. 2nd ed. Tokyo, Japan: Igakushoin; 2003: 36-43), and it has been reported that corneal epithelial damage associated with dry eye distributed across the entire cornea converges on the inferior region of the cornea in the process of symptom improvement (Yokoi N, EBM for dry eye, *Japanese Journal of Clinical Ophthalmology*. 2001;55: 72-85). Therefore, each cornea was divided into three zones for both FL and RB scores in clinical studies of diquafosol ophthalmic solution, with a view to assessing corneal epithelial damage in details and determining treatment response over time and allowing for the evaluation of the treatment response in the central cornea, which is considered particularly clinically important, by obtaining information on “the degree of damage by region.”

PMDA accepted the applicant’s response.

4.(ii).B.(1).2) Efficacy evaluation based on the Definition and Diagnostics of Dry Eye 2006

Although the Definition and Diagnostics of Dry Eye 1995 were used in clinical studies of diquafosol ophthalmic solution, the currently available Definition and Diagnostics of Dry Eye 2006 (“new criteria”) include the presence of subjective symptoms (including visual disturbance) as shown in Table 6. The test procedures have also been revised. For example, according to the old criteria, fluorescein staining of the cornea is graded on a 0-3 scale and a FL score of ≥ 1 is regarded as a positive test result while a FL score of ≥ 3 (summed temporal conjunctiva, cornea, and nasal conjunctiva scores, each on a 0-3 scale, for a maximum score of 9) is regarded as a positive test result in the new criteria. PMDA asked the applicant to explain whether the efficacy of diquafosol in patients meeting the new criteria can also be assured.

Table 6. Definite dry eye based on the Definition and Diagnostics of Dry Eye 2006

1. Subjective symptoms (including visual disturbance)
2. Tear abnormality [either (a) or (b) is met] (a) Schirmer’s I test: ≤ 5 mm (b) Tear film break-up time: ≤ 5 seconds
3. Corneal/conjunctival epithelial damage [(a), (b), or (c) is met] (a) FL score: ≥ 3 (a maximum score of 9)* (b) RB score: ≥ 3 (a maximum score of 9)* (c) Lissamine green staining score: ≥ 3 (a maximum score of 9)* *: Staining of the temporal conjunctiva, cornea, and nasal conjunctiva, with each graded on a 0-3 scale. The scores of the three regions are summed.

Patients are diagnosed as having definite dry eye if all of the above 1, 2, and 3 are met.

The applicant explained as follows:

Subjects enrolled in clinical studies were re-assessed and identified as definite dry eye subjects meeting the new criteria if they have met all of the following conditions: (a) any subjective ocular symptom is present at Week 0, (b) for tear abnormality, Schirmer’s I test score ≤ 5 mm or tear film break-up time ≤ 5 seconds (this condition was ignored because it was part of the inclusion criteria), (c)

as to corneal/conjunctival epithelial damage, the RB scores obtained by assessing both the cornea and conjunctiva, as required by the new criteria, are used and the RB scores in clinical studies (a maximum score of 15) are converted to the scores in the new criteria (a maximum score of 9) (the highest score among the three corneal regions is regarded as the cornea score and then the cornea and nasal and temporal conjunctiva scores are summed) and the subject's converted score is ≥ 3 . As a result, 68.6% of subjects in a late phase II study (194 of 283 subjects) and 92.3% of subjects in a phase III study (264 of 286 subjects) were definite dry eye subjects meeting the new criteria. Any subjective symptom was present at Week 0 in 92.4% of overall subjects (949 of 1027 subjects). As to corneal/conjunctival epithelial damage, even when the converted RB score fails to meet the new criteria, the FL score may meet the new criteria. Thus, it is estimated that there were actually more definite dry eye subjects meeting the new criteria. The mean changes from baseline in the FL and RB scores at Week 4 or early discontinuation in definite dry eye subjects meeting the new criteria were as shown in Table 7 and Table 8. Since the results in definite dry eye subjects meeting the new criteria were also similar to those in the overall population in both the late phase II and phase III studies, it is considered that the revision of the Definition and Diagnostics of Dry Eye does not affect the efficacy evaluation of diquafosol.

Table 7. Mean changes from baseline in FL and RB scores at Week 4 or early discontinuation in definite dry eye subjects meeting the new criteria (Late phase II study)

	FL score				RB score			
	1% diquafosol		3% diquafosol		1% diquafosol		3% diquafosol	
	Definite dry eye	PPS	Definite dry eye	PPS	Definite dry eye	PPS	Definite dry eye	PPS
N	64	93	64	93	64	92	64	93
Mean \pm SD	-1.28 \pm 1.54	-1.34 \pm 1.43	-1.72 \pm 1.36	-1.55 \pm 1.30	-2.00 \pm 1.99	-1.68 \pm 1.94	-2.11 \pm 2.52	-1.71 \pm 2.24
Difference from placebo [95% CI]	-0.36 [-0.85, 0.14]	-0.40 [-0.80, 0.00]	-0.79 [-1.26, -0.33]	-0.60 [-0.98, -0.22]	-0.76 [-1.44, -0.08]	-0.82 [-1.39, -0.26]	-0.87 [-1.64, -0.09]	-0.85 [-1.46, -0.24]

Table 8. Mean changes from baseline in FL and RB scores at Week 4 or early discontinuation in definite dry eye subjects meeting the new criteria (Phase III study)

	FL score				RB score			
	3% diquafosol		HA		3% diquafosol		HA	
	Definite dry eye	FAS	Definite dry eye	FAS	Definite dry eye	FAS	Definite dry eye	FAS
N	131	144	133	142	131	144	133	141
Mean \pm SD	-2.15 \pm 1.66	-2.12 \pm 1.66	-2.11 \pm 1.53	-2.08 \pm 1.52	-3.12 \pm 2.34	-3.06 \pm 2.27	-2.42 \pm 2.10	-2.38 \pm 2.08
Difference from HA [95% CI]	-0.04 [-0.43, 0.35]	-0.03 [-0.41, 0.34]			-0.70 [-1.24, -0.16]	-0.67 [-1.18, -0.16]		

Since, in light of the timing of clinical studies, it was unavoidable to conduct clinical studies in which patients meeting the old criteria were enrolled, and there were no major differences in the results between the population meeting the new criteria and the overall population, PMDA concluded that there is no major problem with evaluating the efficacy of diquafosol ophthalmic solution in dry eye treatment based on the results from clinical studies using the old criteria.

4.(ii).B.(1).3 Efficacy of diquafosol and clinical positioning of diquafosol vs. HA

Since the superiority of diquafosol ophthalmic solution to placebo and the non-inferiority of diquafosol ophthalmic solution to HA ophthalmic solution for the change in the FL score (i.e., an index of corneal epithelial damage) were demonstrated in late phase II and phase III studies,

respectively, PMDA considers that the efficacy of diquafosol in the treatment of corneal epithelial damage associated with dry eye was shown.

On the other hand, diquafosol ophthalmic solution was superior to HA ophthalmic solution for the change in the RB score (i.e., an index of a disruption of the mucin layer covering the corneal and conjunctival epithelium) in the phase III study. PMDA asked the applicant to explain its clinical significance in association with a failure to demonstrate the superiority of diquafosol to HA for subjective symptoms and then show their view on the clinical positioning of diquafosol ophthalmic solution vs. HA ophthalmic solution.

The applicant explained as follows:

Mucins function as a barrier against an external environment, lower the surface tension of tears, lubricate the corneal and conjunctival surfaces, and stabilize the tear film etc. In dry eye patients, a disruption of the mucin layer on the corneal and conjunctival surfaces prevents the tears from spreading evenly over the ocular surface, resulting in an uneven thickness of the tear film, leading to a vicious circle of ocular surface deterioration (Watanabe H. Dry eye associated with abnormal mucus layer. In: Watanabe H, Tano Y, eds. *Practical Ophthalmology* 41. Tokyo, Japan: Bunkodo; 1998; 1: 76-80). Thus, improving mucin deficiency is important for dry eye treatment (Dua HS, Kruse FE. Innovations in the Treatment of dry eye disease: mucin stimulators and hormone replacement. In: Asbell PA, Lemp MA, eds. *Dry eye disease: the clinician's guide to diagnosis and treatment*. New York: Thieme medical publishers; 2006;108-13). Diquafosol was superior to HA for the change in the RB score in the phase III study, showing that diquafosol can improve mucin deficiency, which is not sufficiently treatable with HA, and more effectively treat corneal and conjunctival epithelial damage associated with dry eye. Also, as for the mechanism of action, HA just traps water in the tear film and does not improve the tears and promotes the migration of corneal epithelial cells by directly acting on the corneal epithelium, whereas it is inferred that diquafosol improves corneal and conjunctival epithelial damage by stimulating tear secretion, including mucins, and qualitatively and quantitatively normalizing the tear film. Therefore, diquafosol enables a more causative treatment directed at the underlying pathology of dry eye compared with HA.

Although it is thought that subjective symptoms of dry eye are associated with corneal sensitivity (Belmonte C et al. *Exp Eye Res.* 2004;78: 513-25), there are conflicting reports on this issue. One report states that corneal damage leads to reduced corneal sensitivity and the other states that corneal damage leads to increased corneal sensitivity (De Paiva CS, Pflugfelder SC. *Am J Ophthalmol.* 2004;137: 109-15, Situ P et al. *Invest Ophthalmol Vis Sci.* 2008;49: 2971-6). Objective signs of dry eye are not necessarily correlated with its subjective symptoms. Also, the relationship between mucin deficiency and subjective symptoms is not clear. In a phase III clinical study of diquafosol, the mean change in the total score of subjective symptoms⁷ (mean ± SD) at Week 4 or discontinuation was

⁷ Total score of subjective symptoms (11 items). The severity of each subjective symptom was rated on a 4-point scale (0-3 points).

-2.90 ± 3.89 in the diquafosol group and -2.99 ± 3.92 in the HA group, and the between-treatment difference [95% CI] was 0.09 [-0.84, 1.02], indicating that there was no difference in the improvement of overall subjective symptoms between the two groups. However, when looking at individual symptoms, the mean change in the score for a sensation of heaviness was -0.68 ± 0.80 in the diquafosol group and -0.33 ± 1.05 in the HA group and the between-treatment difference [95% CI] was -0.35 [-0.71, 0.00] ($P = 0.052$), indicating that the improvement effect of diquafosol may be greater than that of HA. On the other hand, with respect to eye discharge, the mean change in the score was 0.05 ± 0.78 in the diquafosol group and -0.27 ± 0.78 in the HA group and the between-treatment difference [95% CI] was 0.32 [0.03, 0.62] ($P = 0.031$), indicating that the improvement effect of diquafosol is smaller, which may be associated with the pharmacological effects of diquafosol because eye discharge is a viscous fluid containing mainly mucins secreted onto the ocular surface.

PMDA asked the applicant to explain their view on clinical positioning of diquafosol ophthalmic solution and HA ophthalmic solution.

The applicant explained as follows:

As a phase III study showed that diquafosol ophthalmic solution was not inferior to HA ophthalmic solution in improving corneal epithelial damage and diquafosol ophthalmic solution was superior to HA ophthalmic solution in improving corneal/conjunctival mucin deficiency, diquafosol is more effective than HA, and there were no major safety problems in any study. Thus, it is considered that diquafosol can be used in virtually all dry eye patients. Based on the mechanism of action and efficacy, if corneal/conjunctival epithelial damage is considered associated with dry eye, the use of diquafosol should be recommended. On the other hand, if corneal/conjunctival epithelial damage is not associated with dry eye but with extrinsic factors, such as trauma, the use of HA should be recommended. Furthermore, in the current medical practice, if HA ophthalmic solution fails to produce sufficient improvement in corneal or conjunctival mucin deficiency, a surgical procedure or autologous serum eye drop has to be used. However, even in such cases, it is expected that patients will be treated with diquafosol eye drop.

PMDA considers as follows:

Taking account of the mechanism of action of diquafosol involving the stimulation of mucin secretion, the applicant's claim that a more causative treatment directed at the underlying pathology of dry eye is possible with diquafosol ophthalmic solution as compared with an existing treatment, i.e. HA ophthalmic solution is acceptable. However, there is insufficient clinical evidence supporting this claim and the positioning of diquafosol needs to be further defined after collecting information on long-term QOL improvement and contribution to remission via post-marketing surveillance etc. Especially, although the applicant claims that diquafosol can be used also in patients for whom a surgical procedure or autologous serum eye drop would otherwise be indicated, such patients with severe cases were not included in clinical studies and this is pure supposition at present. Therefore, it is necessary to fully assess the usefulness of diquafosol in patients with severe cases (especially,

patients with severe mucin deficiency [a high RB score] in whom the distinctive effects of diquafosol can be shown) via post-marketing surveillance.

4.(ii).B.(2) Safety

4.(ii).B.(2).1) Local ocular adverse events

The applicant explained the safety profile of diquafosol ophthalmic solution as follows:

The incidences of adverse events and adverse drug reactions with 3% diquafosol were 35.2% (102 of 290 subjects) and 16.6% (48 of 290 subjects), respectively, in the short-term treatment population and 62.2% (227 of 365 subjects) and 22.5% (82 of 365 subjects), respectively, in the long-term treatment population and most of the events classified as adverse drug reactions were local ocular events in both populations.

Local ocular adverse events with a high incidence were eye irritation (7.3%) (48 of 655 subjects), eye discharge (6.6%) (43 of 655 subjects), and conjunctival hyperaemia (7.3%) (48 of 655 subjects) and all of these events were mild in severity except for moderate conjunctival hyperaemia in 1 subject. Three events of eye irritation, 2 events of eye discharge, and 7 events of conjunctival hyperaemia led to study discontinuation, but most subjects were able to continue treatment. The majority of these events occurred by Week 4 and the incidences did not increase with prolonged treatment.

In a HA-controlled phase III study, although the incidences of adverse events of eye irritation and eye discharge were higher in the diquafosol group (6.3% and 2.8%, respectively, in the diquafosol group, 0.7% and 1.4%, respectively, in the HA group), most subjects were able to continue treatment, and the events resolved during diquafosol treatment or soon after treatment completion/discontinuation. Therefore, there should be no problem with the safety and tolerability of diquafosol ophthalmic solution.

PMDA considers as follows:

Following topical ocular administration of diquafosol ophthalmic solution, no clinically relevant systemic adverse events or adverse drug reactions occurred. Although the incidences of local ocular adverse events and adverse drug reactions were higher with diquafosol compared with a related product, most of them were mild in severity and reversible. Therefore, there should be no major safety concern about diquafosol at present, but the safety of diquafosol in routine clinical settings needs to be further investigated. Especially, eye discharge may have developed/worsened due to the pharmacological effects of diquafosol [see 4.(ii).B.(1).3) Efficacy of diquafosol and clinical positioning of diquafosol vs. HA], and its occurrence and relationship to diquafosol treatment, etc. need to be investigated with priority via post-marketing surveillance. As the cause of dry eye varies, it is also necessary, via post-marketing surveillance, to fully investigate the safety of diquafosol in patients with dry eye caused by contact lense wear or eye surgery, and patients with potential risk factors, e.g., patients with allergic conjunctivitis, who were not enrolled in clinical studies.

4.(ii).B.(2).2) Safety during concomitant use with other eye drops

Although the concomitant use of other eye drops was prohibited in clinical studies of diquafosol ophthalmic solution, the diquafosol product may be used concomitantly with other eye drops after the market launch. PMDA asked the applicant to discuss safety and efficacy during concomitant use.

The applicant explained as follows:

Eye drops that are potentially used concomitantly with diquafosol ophthalmic solution include timolol (an antiglaucoma drug), levofloxacin and ofloxacin (antibacterial agents), fluorometholone and betamethasone (corticosteroids), pranopfen and azulene (nonsteroidal anti-inflammatory drugs), FAD (a corneal therapeutic agent), and HA. From a pharmacokinetic point of view, diquafosol is considered to be rapidly metabolized to endogenous substances after topical ocular administration and there is no report that these eye drops affect the enzymes involved in the metabolism of diquafosol (ecto alkaline phosphodiesterase I, etc.); conversely, there is no report suggesting that diquafosol and its metabolites affect the pharmacokinetics of concomitant eye drops. Thus, there should be little possibility that drug interactions occur during concomitant use. From a pharmacodynamic point of view, although timolol lowers intraocular pressure by blocking β -receptors, diquafosol has no affinity for β receptors (4.2.1.1-017) and there is also no report that timolol shows affinity for the P2Y₂ receptor. As for other eye drops, there should be little possibility that drug interactions occur during concomitant use because of clearly different mechanisms of action from diquafosol. Taking account of these findings, after the market launch, as long as an adequate interval is allowed between products, the concomitant application of diquafosol ophthalmic solution with other eye drops will not affect safety or efficacy.

PMDA accepts the above response at present, but as there are no data on the concomitant use with other eye drops, it is necessary to confirm its safety via post-marketing surveillance.

4.(ii).B.(3) Efficacy and safety in patients with dry eye due to Sjogren's syndrome or Stevens-Johnson syndrome

The applicant explained about treatment outcomes in patients with Sjogren's syndrome (SS) or Stevens-Johnson syndrome (SJS) as follows:

Eighty-one patients with SS (18 patients in an early phase II study, 16 patients in a late phase II study, 36 patients in a phase III study, 11 patients in long-term treatment studies) were assigned to the 3% diquafosol groups across all clinical studies. FL and RB scores in SS and non-SS patients treated with 3% diquafosol ophthalmic solution in the early phase II, late phase II, and phase III studies were as shown in Table 9. There were no major differences in efficacy between the two subgroups across all studies. Regarding safety, pooled data from all studies were analyzed. As a result, although the incidence of eye irritation was higher in SS patients (14.8% [12 of 81 patients]) than in non-SS patients (6.3% [36 of 574 patients]), the incidence of overall adverse events was 51.9% in SS patients (42 of 81 patients) and 53.1% in non-SS patients (305 of 574 patients) and the incidence of adverse drug reactions was 23.5% in SS patients (19 of 81 patients) and 21.1% in non-SS patients (121 of 574

patients). No differences between the subgroups were observed and there were no tolerability problems in SS patients as well.

Table 9. Mean changes from baseline in FL and RB scores at Week 4 or early discontinuation in SS and non-SS patients (FAS)

	Early phase II study		Late phase II study		Phase III study	
	SS patients	Non-SS patients	SS patients	Non-SS patients	SS patients	Non-SS patients
N	18	30	16	79	36	108
FL score	-1.56 ± 2.12	-1.53 ± 1.70	-1.19 ± 1.60	-1.59 ± 1.22	-1.81 ± 2.07	-2.22 ± 1.50
RB score	-1.78 ± 3.04	-1.07 ± 1.64	-1.56 ± 3.08	-1.68 ± 2.06	-2.72 ± 2.55	-3.17 ± 2.17

Mean ± SD

Two patients with SJS were enrolled in a long-term treatment study (Study 00890603) and completed treatment up to Week 28. As to efficacy, both patients showed improvement in FL and RB scores after treatment with diquafosol ophthalmic solution (Subject Number 895-05-02, change in FL score at Week 28 was -2, change in RB score at Week 28 was -5; Subject Number 895-13-02, change in FL score at Week 28 was -3, change in RB score at Week 28 was -3). Regarding safety, conjunctival oedema and asthenopia (2 events each) and meibomianitis, foreign body sensation in eyes, photophobia, eye pain, vision blurred, ocular discomfort, eye discharge, and conjunctival hyperaemia (1 event each) were reported by Subject Number 895-05-02; and nasopharyngitis (2 events) and corneal disorder, conjunctival hyperaemia, and eye irritation (1 event each) were reported by Subject Number 895-13-02. A causal relationship to study drug could not be denied for eye irritation and conjunctival hyperaemia reported by Subject Number 895-13-02, but both events were mild in severity and resolved without treatment discontinuation.

Based on the above, the efficacy and safety of diquafosol ophthalmic solution in the treatment of dry eye due to SS or SJS were demonstrated.

PMDA considers as follows:

The efficacy of diquafosol ophthalmic solution in patients with SS can be expected because a certain number of patients with SS were enrolled in each study and there were no major differences in the changes in staining scores between SS and non-SS patients. Although only 2 patients with SJS were enrolled into clinical studies, in light of the rarity of the disease, it is understandable that patient enrollment was very difficult. Both patients showed improvement in FL and RB scores and their results demonstrated a similar trend to those of the overall population. Thus, a certain level of efficacy can be expected. The safety profiles in SS and SJS patients also are not different from that in the overall population and there is no clinically relevant problem at present. However, as the limited number of cases were studied for both diseases, it is necessary to continue to investigate the safety and efficacy of diquafosol ophthalmic solution in these patients via post-marketing surveillance.

4.(ii).B.(4) Indication

PMDA considers as follows:

Although the proposed indication was “corneal and conjunctival epithelial damage associated with dry eye (including Sjogren’s syndrome and Stevens-Johnson syndrome),” the Definition and Diagnostics

of Dry Eye 2006 define dry eye as a chronic multifactorial disease of the tears and corneal and conjunctival epithelium and do not distinguish dry eye patients according to the presence or absence of primary disease for diagnosis and treatment. Although dry eye due to SS or SJS is generally severe, as described above, there are no substantial differences in treatment response according to primary disease. Therefore, diquafosol ophthalmic solution should be indicated for the treatment of “dry eye” in patients meeting the dry eye diagnostic criteria, regardless of its etiology.

The indication statement for diquafosol ophthalmic solution will be finalized, taking account of comments from the Expert Discussion.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA’s conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based compliance inspection and data integrity assessment was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, there were no particular problems. Thus, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

2. PMDA’s conclusion on the results of GCP on-site inspection

GCP on-site inspection was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application (5.3.5.1-001, 5.3.5.1-002, 5.3.5.1-003, 5.3.5.2-002, 5.3.5.2-003). As a result, the following findings were noted at some trial sites: despite the fact that investigators etc. gave a supplementary explanation when obtaining consent from a subject, the informed consent form was not dated by the subject; and a subject who met the exclusion criteria because of the use of a protocol-specified prohibited concomitant medication was enrolled in the study. PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application dossier.

IV. Overall Evaluation

Based on the submitted data, it is considered that the efficacy of diquafosol ophthalmic solution in dry eye treatment has been demonstrated and its safety is acceptable in view of its observed benefits. As diquafosol has a novel mechanism of action, it offers a new option for dry eye treatment and has clinical significance. The proposed indication statement should be changed. Although it is considered that there are no particular safety problems at present, it is necessary to continue to investigate the safety of diquafosol ophthalmic solution via post-marketing surveillance.

This application may be approved if it can be concluded based on comments from the Expert

Discussion that there are no particular problems.

Review Report (2)

February 8, 2010

I. Product Submitted for Registration

[Brand name]	Moistear Ophthalmic Solution 3%
[Non-proprietary name]	Diquafosol Sodium
[Name of applicant]	Santen Pharmaceutical Co., Ltd.
[Date of application]	May 30, 2008

II. Content of the Review

The Expert Discussion and subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined below.

The expert advisors for the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for registration, in accordance with the provisions of the “Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency” (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

(1) Indication

PMDA’s conclusion that Sjogren’s syndrome and Stevens-Johnson syndrome should not be specifically mentioned in the proposed indication statement and the use of diquafosol ophthalmic solution should be considered for patients meeting the dry eye diagnostic criteria, regardless of its etiology, was supported by the expert advisors. PMDA concluded that the indication should be changed to “dry eye.”

(2) Post-marketing surveillance

PMDA concluded that it is necessary to accumulate the information on the safety of diquafosol ophthalmic solution in routine clinical settings, etc. and requested the applicant to design post-marketing surveillance so that the safety and efficacy of long-term treatment with diquafosol can also be investigated.

The applicant explained as follows:

A drug use-results survey with a 2-month observation period will be conducted to collect the information on the use of diquafosol ophthalmic solution in patients with severe cases or pediatric patients, which was lacking or unavailable from clinical studies, and investigate the effects of concomitant medications on the safety and efficacy of diquafosol, etc. Furthermore, a long-term, special drug use-results survey with a 1-year observation period will be conducted to investigate the effects of diquafosol ophthalmic solution on remission of symptoms and QOL as well.

PMDA considers that these surveys should be conducted promptly and the information on the obtained results should be provided to clinical practice appropriately.

III. Overall Evaluation

As a result of the above review, PMDA concludes that the product may be approved after modifying the indication, and dosage and administration statements as shown below. The re-examination period is 8 years, neither the drug substance nor the drug product is classified as a poisonous drug or a powerful drug, and the product is not classified as a biological product or a specified biological product.

[Indication]

Dry eye

[Dosage and administration]

The usual dose is one drop instilled into the eye six times a day.

(Appendix)

The summary of the submitted data and the outline of review by PMDA regarding the drug master file (DMF) for Diquafosol ophthalmic solution 3% (DMF No. 220MF10109)

[Brand name] Diquafosol Sodium
[Non-proprietary name] Diquafosol Sodium
[Name of submitter] Yamasa Corporation
[DMF No.] 220MF10109

Summary of the submitted data on the drug substance

The drug substance is a white crystalline powder and its physiochemical properties have been determined by description, solubility, hygroscopicity, melting point/thermal analysis, pH, optical activity/optical rotation, dissociation constant, partition coefficient, and crystalline polymorphism. The drug substance is hygroscopic and has no polymorphs.

The drug substance is produced using [REDACTED] and [REDACTED] as starting materials. The manufacturing process consists of Step 1 and Step 2 ([REDACTED]), Step 3 (synthesis of [REDACTED]), Step 4 (synthesis of [REDACTED]), Step 5 (synthesis and purification of [REDACTED]), and Step 6 (packaging). Step [REDACTED] and Step [REDACTED] have been defined as critical steps and [REDACTED] has been defined as a critical intermediate.

The chemical structure of the drug substance has been elucidated by elementary analysis, infrared (IR) spectrophotometry, mass spectrometry, nuclear magnetic resonance spectrometry (¹H-NMR, ¹³C-NMR, ³¹P-NMR), and ultraviolet (UV) spectrophotometry. As for impurities in the drug substance, related substances, a residual solvent (ethanol), and degradation products (heat and light) have been analyzed.

The proposed specifications for the drug substance are description (color and state), identification (IR spectrum, sodium, optical rotation), purity (heavy metals, arsenic, related substances, residual solvent [ethanol]), water content, and assay (content) (HPLC). A specification limit of ≤ [REDACTED] ppm for purity (heavy metals) and a specification limit of ≤ [REDACTED] ppm for purity (arsenic) have been proposed. The proposed specification limits for related substances are ≤ [REDACTED]% for Related Substance A, ≤ [REDACTED]% for individual related substances, and ≤ [REDACTED]% for total related substances. There should be no safety problem with Related Substance A because it is a metabolite of Diquafosol Sodium in ocular tissue and blood, and is an endogenous substance and is metabolized to Related Substance B. The proposed specification limit for ethanol was ≤ [REDACTED] ppm, which has been changed to [REDACTED] ppm in the course of the regulatory review.

In order to assess the stability of the drug substance, long-term testing (5 ± 3°C, dark place, polyethylene bag/fiber drum, 36 months) and accelerated testing (25 ± 2°C/60 ± 5%RH, dark place, polyethylene bag/fiber drum, 6 months) were performed using 3 commercial scale lots; and stress

testing (heat [80°C, open and closed containers, 14 weeks]), stress testing (light [25°C/60%RH, polyethylene bag, not less than 1.2 million lx·hr + not less than 200 W·h/m²]), and stress testing (light [25°C/60%RH, quartz cell, not less than 1.2 million lx·hr + not less than 200 W·h/m²]) were performed using 1 commercial scale lot. The attributes tested include description (color and state), identification (IR), purity (related substances), water content (%), assay, and pH.

Under the long-term storage and accelerated conditions, there were slight increases in Related Substance A as a degradation product (after 36 months of long-term storage, up to ██████%; after 6 months of accelerated storage, up to ██████%), but no changes with time for other attributes tested. Under the stress condition (heat), there were increases in Related Substance B, Related Substance C, and Related Substance A as degradation products. Under the stress conditions (light), no changes occurred. As the drug substance is hygroscopic, a humidity-protecting container closure system is proposed and no stress testing (humidity) has been performed. Based on the long-term storage data, a re-test period of 3 years has been proposed for the drug substance when stored in a humidity-protecting container closure system at 5 ± 3°C.

Outline of drug substance review by PMDA

While Diquafosol Sodium is hygroscopic, no stress testing (humidity) has been performed. PMDA asked the applicant to explain the possibility that related substances as degradation products increase as water content increases and provide a justification for the proposed upper specification limit of ██████% for water content.

The drug master file registrant explained as follows:

In stress testing (heat + humidity [40°C/75%RH, 6 months], polyethylene bag/cardboard drum, 2 commercial scale lots) and accelerated testing (25°C/60%RH, 36 months, polyethylene bag/cardboard drum, 2 commercial scale lots) performed as preliminary studies, as water content increased, Related Substance A increased transiently (after 1 month of storage at stress condition, up to ██████% [water content, ██████%]; after 6 months of accelerated storage, up to ██████% [water content, ██████%]) and then decreased. Related Substance C, which is considered a degradation product of Related Substance A, increased in a water content-dependent manner (after 6 months of storage at stress condition, up to ██████% [water content, ██████%]; after 36 months of accelerated storage, up to ██████% [water content, ██████%]). However, based on the increase and decrease over time, it was considered that Related Substance A and Related Substance C would not exceed their proposed upper specification limits of ██████% and ██████%, respectively, at ██████% of water content. Therefore, the proposed upper specification limit for water content (██████%) is justified.

PMDA accepted the above response and concluded that the proposed specifications, test procedures, storage conditions, and re-test period for the drug substance are acceptable.